A STUDY OF SOME FACTORS INFLUENCING THE DISTRIBUTION OF CARBON DIOXIDE IN THE BODY

Sourindra Mohan Ghosh

Department of Physiology, University of Edinburgh

Thesis presented for the

Degree of Doctor of Philosophy,

University of Edinburgh.

1954

TABLE OF CONTENTS

		Page
Acknowledgements		
General Introduction		1
Part I.	Diffusion and Solubility of Carbon Dioxide in Fats ..	11
Part II.	Exchangeability of Carbonate with Phosphate in Bones ..	47
Part III.	Respiratory Adaptation to Carbon Dioxide Administered in the Inspired Air at a Fixed Rate	76
Abstract		111
References		

ACKNOWLEDGEMENTS

This work was undertaken in the Department of Physiology, University of Edinburgh. For the laboratory facilities I wish to thank Professor D. Whitteridge. The problems were suggested by the late Dr. Philip Eggleton who supervised the work until his untimely death in October, 1954. Dr. Eggleton's views and advice were always given freely and even though troubled by illhealth he took an interest in this work until the end; for this I am greatly indebted to him.

Dr. Dewar and Dr. Hastewell of the Department of Physiology at Edinburgh University, I wish to thank for their help.

GENERAL INTRODUCTION

Carbon dioxide is of ubiquitous distribution in the animal body. It originates in the tissue cells as an end product of metabolism. But, by no means, is it a useless waste in the same sense as are the end products of nitrogenous metabolism. In many ways an optimum concentration of carbon dioxide in the body is physiologically desirable. The problem of the animal body is not complete elimination but maintenance of an optimum distribution of carbon dioxide in its various tissues.

In the cells of the tissues and outside carbon dioxide reacts with water to form an acid which on dissociation becomes an integral part of the electrolyte structure of the body fluids. The distribution and movement of carbon dioxide in the aqueous fluids of the body including blood is largely a question of the electrolyte distribution and the influence of haemoglobin on such a distribution in the presence and lack of oxygen.

The chemical equilibrium and kinetics of the reactions of carbon dioxide in various aqueous systems of biological interest, specially blood, have been very thoroughly studied during the first four decades of the present century by a large number of investigators, and have been reviewed by Van Slyke (1926), Henderson (1928), and Roughton (1935)(1943). As a result of these studies, the

differences in the distribution of carbon dioxide between the red blood cells and plasma, between plasma and extracellular fluid and, in some tissues, between intra and extracellular fluids can be predicted and explained fairly satisfactorily by the application of the laws of Mass Action and Gibbs-Donnan equilibrium (Hastings, 1940).

There are, however, two non-aqueous phases in the body, namely the fat and the mineral matter of the bones. A scheme of looking at the animal body as composed of four principal compartments is shown in Fig. 1. Each of these compartments differs in some respect in its relationship to carbon dioxide. The intracellular compartment is the site of its production. The extracellular fluid is the vehicle of its transport and distribution. In the bones carbon dioxide is combined with solid matter. In the fat it has no chemical affinity.

The extracellular fluid is in contact with each of the other compartments and with air in the alveolar spaces of the lungs. The researches of Haldane and Priestley (1905) have shown that the pulmonary ventilation rate of a subject is so regulated as to ensure a constancy of the partial pressure of carbon dioxide in the alveolar spaces of the lungs. The whole system may be looked upon as behaving like a tonometer in equilibrium with a constant tension of carbon dioxide in the alveoli

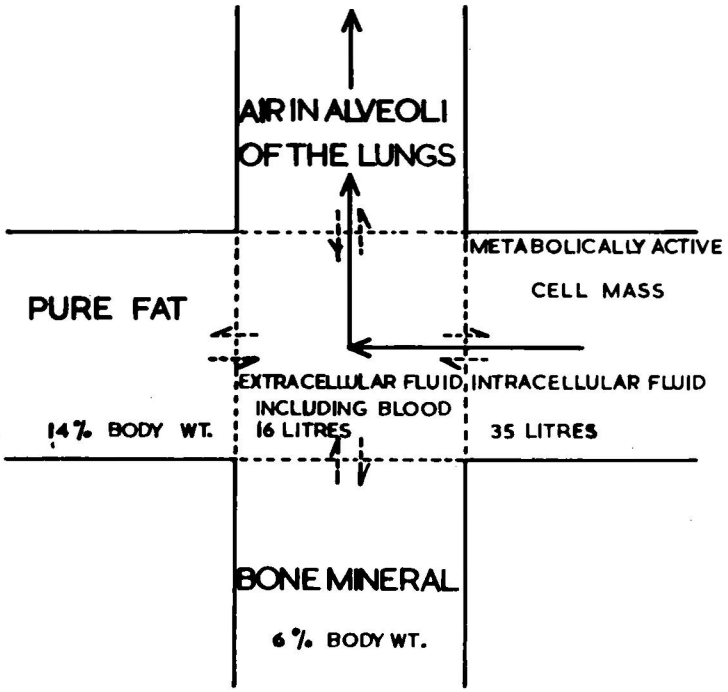

Fig. 1.

A schematic division of the animal body into four compartments*.

*The figures shown in the diagram are those corresponding to a normal man. In such a body the fat, which is mainly intracellular, amounts to 14%, and the bone mineral to 6%, of the body weight (Keys and Brozac, 1953). The quantities of intra and extracellular fluids are respectively 35 and 14 litres (Marriott, 1947); but to the extra cellular component there is added two litres which corresponds to the volume of red cells, making up a total volume of 16 litres as shown in the diagram.

of the lungs. The equilibrium is of the steady state/type, with a net movement of carbon dioxide from the tissue cells through the extracellular fluids into the lungs, and from the lungs into the outside atmosphere at the rate of its production in the body.

The problems investigated in the present work concern three situations in the diagram. The first part deals with the diffusion of carbon dioxide in fat, the second part with a probable mechanism of the exchange of carbonate between bone mineral and extracellular fluid, and the third part with the response of the system as a whole to the introduction of extra carbon dioxide into the lungs.

So far as fat is concerned carbon dioxide has no chemical affinity for it; therefore a knowledge of its solubility, diffusion coefficient, and partial pressure completely describes its behaviour in fat. Its solubility in fats has been measured by Vibrans (1935) and by Schaffer and Haller (1943); but there has not been seemingly any measurement of its rate of diffusion in fats. When one considers that the adipose tissues are poorly supplied with blood and that the capillary bed available for gaseous exchange may be only one-third or even less of that found in the most poorly supplied muscle (Gersh and Still, 1945), the question of its diffusibility in fat assumes an importance. Accordingly, measurement of the diffusion of carbon dioxide in fats forms a part of the present

investigation.

By far the largest store of carbon dioxide in the body is in the solid mineral matter of the bones. According to an estimate of Shohl (1939), a human skeleton contains 7·4 gm. equivalents of carbonate, i.e. 82 litres of carbon dioxide. The potentiality of the bones to influence the distribution of carbon dioxide in the body fluids is obvious. Investigations by Ferguson, Irving and Plewes (1929), by Irving, Ferguson and Plewes (1930) and by Freeman and Fenn (1953) suggest that the carbon dioxide stores of the animal skeleton are labile enough to respond to changes in the concentration of carbon dioxide in the alveolar air of the lungs. Carbon dioxide of the bone is not in a state directly dissociable in response to changes of pressure in the gas. The changes in the composition of the bones in the above investigations are attributible to changes in the level of plasma bicarbonate resulting from changes in alveolar carbon dioxide pressure.

An important question thus arises as to how a change in the level of plasma bicarbonate induces a change in the composition of the bone.

There have been several other investigations in which the experimental procedure has produced proportionately greater changes in the carbonate fraction than in the fractions of calcium, and phosphate (Huggins, 1937). However, no serious attempt

has been made to account for the fact that the observed changes in the carbonate, phosphate and calcium rarely follow the proportion in which they are usually present in the bones. Moreover, the changes in carbonate and phosphate may not be in the same direction. In the experiments of Freeman and Fenn (1953) the bones of rats made to live in an atmosphere of 10% carbon dioxide for some days showed increase in carbonate but decrease in phosphate contents. Sobel, Rockenmacher and Kramer (1945) have observed in experimental rickets of rats a reciprocal relationship between serum inorganic phosphorus and bone carbonate.

An appraisal of the above evidence, which will be discussed more fully at a later stage, has appeared to suggest that one of the mechanisms of the participation of bones in exchanges with body fluids may be a form of anion exchange.

In an attempt to test this hypothesis of anion exchange between bone and surrounding fluid, observations have been made to investigate whether the two main anions of the bone, the phosphate and the carbonate are to any extent exchangeable if fresh preparations of frog bones are placed in solutions of bicarbonate and phosphate. An observation of M.G. Eggleton (1933) that frog bones placed in suitably prepared Ringer solutions can take up or give out considerable quantities of phosphate, suggested the above approach to the

problem.

A fair measure of agreement exists that bone mineral has an apatite structure $n.Ca_3(PO_4)_2.CaX$ where X is CO_3 or possibly O, Cl_2, SO_4, F, etc. (Newton, 1939). Neuman, Neuman, Main, O'Leary and Smith (1950) claim to have demonstrated that OH, F, and HCO_3 ions compete equally for the X position in crystals of ashed bone powder.

The investigations which form the second part of the present work intend to show that in the substance of the fresh bone there exist anion positions for which the bicarbonate and the phosphate ions can compete in, in vitro, experiments of some hours duration.

The preceding account refers to purely physicochemical aspects of the distribution of carbon dioxide in the body. The maintenance of an equilibrium of carbon dioxide in the body is, however, an essentially physiological function.

The rate of production of carbon dioxide in the body is determined by the metabolic needs of the moment; and it is the rate of its elimination which is physiologically regulated to produce a steady state of its equilibrium in the body. The final stage of the elimination of carbon dioxide from the body is from the alveolar spaces of the lungs, and so the amount of carbon dioxide leaving the body depends on its concentration in the alveolar air and the rate of pulmonary ventilation.

It has been shown by Haldane and Priestley (1905) that the physiological regulation of pulmonary ventilation tends to ensure a constancy of the partial pressure of carbon dioxide in the alveolar air.

In their experiments Haldane and Priestley (1905) inhaled air containing several different percentages of carbon dioxide and showed that there was very little change in the partial pressure of alveolar carbon dioxide. In those of their experiments in which they measured the alveolar carbon dioxide they did not measure the ventilation rate but derived it by calculation, assuming that they were maintaining a steady state of carbon dioxide equilibrium during the experiments.

When similar experiments were repeated, Campbell, Douglas, Haldane and Hobson (1913) realised that on breathing air enriched with carbon dioxide there would follow a period of adjustment during which the elimination of carbon dioxide from the body would be temporarily retarded, and an amount of carbon dioxide would remain dammed back in the body to be given out when ordinary air was again inhaled. Trying to investigate how long the periods of unsteadiness lasted, they found that the periods were probably much longer than they had thought.

Physiologists who have experimented on the effects of carbon dioxide on pulmonary ventilation

have been mainly interested to measure the ventilatory response after it has reached an apparently steady level. Not much attention, however, has been paid to the periods of adjustment that occur at the beginning and immediately after the administration of carbon dioxide.

Padget (1928), Dripps and Comroe (1947), Duncan-Weatherley (1952) and Lambertsen, Kough, Cooper, Emmel, Loescheke, and Schmidt (1952) have found or considered that a maximum ventilatory response is reached in 2 to 10 minutes depending on the strength of the carbon dioxide mixture inhaled. It will be discussed later that a maximum in the ventilatory response does not necessarily mean that the steady state of carbon dioxide elimination from the body has been regained, especially if the air mixture contains high percentages of carbon dioxide. In fact Campbell, Douglas and Hobson (1914) have found that the respiratory quotient of a subject inhaling 3·5% carbon dioxide in air did not return to normal and remained low even up to 1¼ hours.

As regards the magnitude of the amount of carbon dioxide likely to be retained and later given out, the only attempt that has been made to make an estimation was by Adolph, Nance and Shilling (1928). The estimate varied from 400 to 2100 c.c. of carbon dioxide with one exceptionally high value of 4900 c.c. The subjects inhaled a 5% mixture of carbon dioxide in air.

There is considerable uncertainty, firstly about the duration of the period of adjustment, and secondly about the amount of carbon dioxide retained, and later released. In the experiments of the third part of the present work, the steady state of carbon dioxide equilibrium of the body has been subjected to the disturbing influence of the addition of carbon dioxide to the inspired air and to its subsequent withdrawal. By studying the effects of the above procedure on the pulmonary ventilation rate and on the rate of elimination of carbon dioxide in the expired air, an attempt has been made to find out how soon the steady state is regained and how much carbon dioxide is retained or released from the body during the adjustment.

To study the respiratory effects of carbon dioxide a closed circuit method, in which a subject rebreathes from a bag, has occasionally been used (Peabody, 1915; Davies, Brow and Binger, 1925). In such a system the concentration of carbon dioxide in the bag and its tension in the tissues rise continually and the subject does not reach a steady state. In the experiments of Haldane and Priestley (1905) and in those of Campbell et al (1913), and Campbell et al (1914), the subject sat inside a closed chamber into which a measured volume of carbon dioxide was introduced to produce initially a desired concentration of carbon dioxide in the air inside. As the chambers were of large capacity,

the concentration of carbon dioxide did not change materially thereafter. More commonly, however, the percentage of carbon dioxide in the inspired air has been maintained at a fixed level by making the subject inhale from a reservoir containing a gas mixture of a desired composition.

A subject inhaling a fixed percentage of carbon dioxide in air would reach a steady state after some time. But the condition of the experiment would not be comparable to the state of a subject responding to extra carbon dioxide produced in his body. In the latter case the amount of carbon dioxide required to be eliminated in the expired air, to maintain a steady state, is independent of the rate of ventilation. With the object of creating a condition similar to that produced by an increase in endogenous carbon dioxide, a new procedure of administering carbon dioxide has been adopted in the present study, by administering it at a fixed rate per unit time rather than by fixing its percentage in the inspired air.

PART I

DIFFUSION AND SOLUBILITY OF CARBON DIOXIDE IN FATS

INTRODUCTION

This part of the work describes a measurement of the solubility and coefficient of diffusion of carbon dioxide in fats. The primary object was to measure the coefficient of diffusion but, since the method employed allowed the solubility to be measured at the same time, both measurements have been made. The data obtained have been applied to assess the distribution and movement of carbon dioxide in body fats.

There have been several measurements of the diffusion coefficient of carbon dioxide in animal tissues (Krogh, 1919; Fenn, 1928; and Wright, 1934), and in other media such as water, rubber and gelatin (Hufner, 1897; Daynes, 1920; Wright, 1934; and Hagenbach, 1898). But it has not been measured in pure fats.

The solubility of carbon dioxide in fats and oils has been measured by Vibrans (1935) and by Schaffer and Haller (1943). Their measurements on animal fat (lard) were made at 45°C and 40°C. The present measurements have been made at 37°C and lower temperatures.

Davidson, Eggleton and Foggie (1952) have recently measured the solubility and coefficient of diffusion of nitrogen, hydrogen, and oxygen in a number of solvents including olive oil and lard.

In the present work their method has been extended to make measurements of a similar nature with carbon dioxide. Measurements have been made of its solubility and coefficient of diffusion in a sample of lard and a sample of olive oil.

METHODS

The apparatus and technique used in the present investigation were essentially the same as employed by Davidson et al (1952). But compared to the gases studied by them carbon dioxide was very much more soluble in the solvents studied, and the pressure changes were too great to be measured by a water manometer. This difficulty has been overcome by using mercury in the manometer instead of Brodie fluid.

The general arrangement of the apparatus used in these experiments is shown schematically in Fig. 2. The closed vessel A contains the oil or fat on which the measurements are made. This can be connected through taps T_1 and T_2 to a vacuum pump, capable of maintaining a vacuum of 10^{-3} m.m. of Hg. The vessel A is charged with the substance to be tested, then the vacuum pump is used to evacuate the air space and to rid the substance of any dissolved gases. The initial evacuation may be complicated by frothing inside the apparatus and thus it is necessary to do a preliminary evacuation by connecting the diffusion vessel to the pump through a splash bulb. After the frothing has ceased, the splash bulb is removed and the pump and the diffusion vessel are directly connected to the apparatus, as shown in the diagram, for the final stage of the evacuation. This stage lasts for half to one hour.

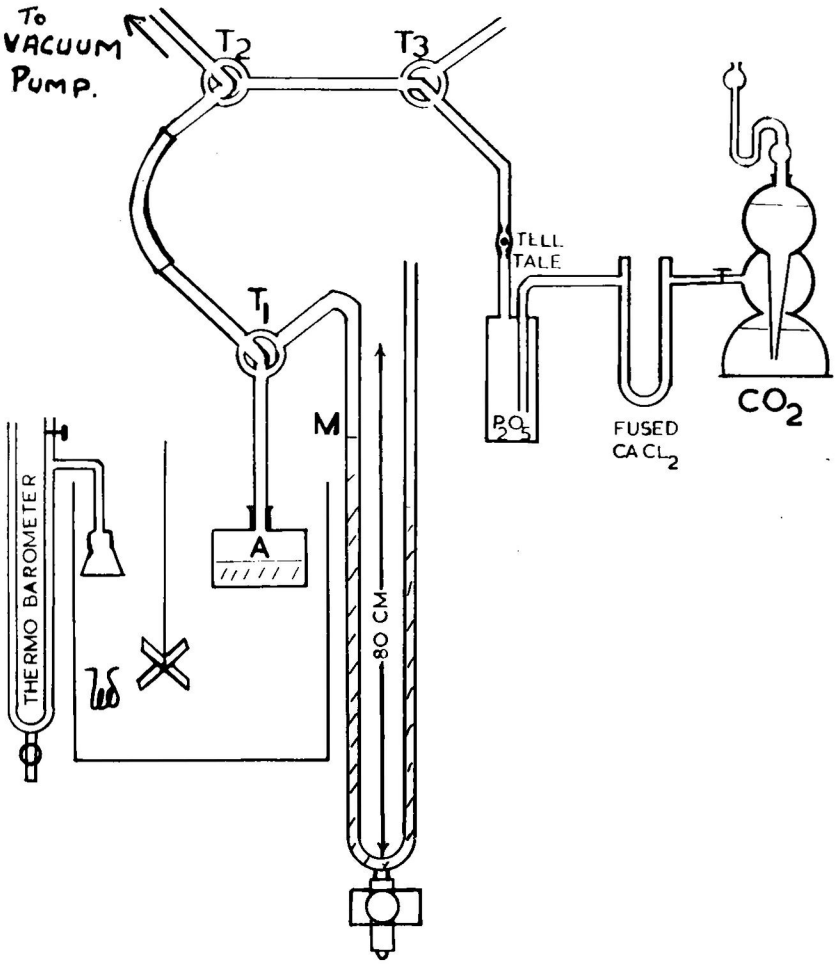

Fig. 2.

Diagram of apparatus for measuring the coefficient of diffusion and the solubility of carbon dioxide in a fat.

After the evacuation of vessel A has been completed, the tap T_2 is turned so that the vessel A is in communication with the source of carbon dioxide. As the gas commences to flow, a glass bead, used as a 'tell-tale', shows a momentary movement. A stop watch is started at this moment of movement of the 'tell-tale', this being taken as the zero time for the experiment. Two seconds after the starting of the stop watch, the tap T_1 is turned and the manometer is brought into communication with the space inside the vessel A. The gas space in the closed limb of the manometer is always pre-charged with carbon dioxide at the very beginning of each experiment, so that all the gas space contains carbon dioxide. A quantity of the gas is thus entrapped in a closed space above the layer of liquid which has been made gas free. The volume of this gas space is maintained constant by maintaining the level of mercury in the closed limb of the manometer at a suitably fixed level, M. This is done by adjustment of the screw clamp at the base of the manometer.

As the gas diffuses into the liquid, its pressure in the gas phase falls. The pressure readings are recorded from the level of the mercury in the open limb of the manometer at suitable intervals of time. This stage of the experiment lasts for half to one hour and furnishes a series of values of the pressure in the gas phase during the

process of diffusion. The first reading is taken within 30 seconds of starting the stop watch. The subsequent readings are taken at intervals progressively increasing from one to five minutes, because with time the diffusion becomes slower.

The next step is to find the final equilibrium pressure between the gas and the liquid. For this purpose the diffusion bottle is shaken without disturbing its connection with the manometer. The shaking is repeated till successive readings of the pressure show no further fall of pressure. But, as has been pointed out by Davidson et al (1952), a pressure reading taken immediately after the final shaking may not be the true equilibrium pressure. This may be due to the shaking producing a supersaturated condition and thus two readings are taken; one at one minute and another at thirty minutes after the cessation of shaking, so that the effect of super-saturation, if any, can be avoided and allowed for in arriving at the true value of the equilibrium pressure.

The diffusion vessel is finally detached from the apparatus below tap T_1, and weighed to determine the weight of the oil inside, so that knowing its density the volume could be calculated.

The vessels used in the experiment had previously been weighed empty before putting in the oil, and their cross-sectional areas had been determined by charging them with successively increasing

amounts of mercury. The change in the height of the mercury column was measured with a travelling microscope, so that the cross-sectional area of the vessel could be estimated at varying levels. It is required by the theory developed later that the vessels should be of uniform cross-section over the portion which will be occupied by the solvent during an experiment.

The two vessels selected for the present experiments had the following cross-sectional areas at four different levels covering a height of about 1·5 cm. from the bottom.

Vessel 1	Vessel 2
16·44 sq.cm.	16·39 sq·cm.
16·98 "	16·72 "
16·50 "	16·51 "
16·52 "	16·19 "

The other data to be known about the apparatus is the total volume of the enclosed space within the diffusion vessel and the closed limb of the manometer. To find this, the vessel A, the stem of the manometer to which it is mounted, the bore of tap T_1 and the closed limb of the manometer up to an arbitrary known point on it, are filled with mercury and weighed. The volume of the manometer tubing per cm. length is separately determined so that whatever level of the manometer is selected

as the constant volume mark for an experiment, the total volume of the enclosed space can be calculated.

Constancy of temperature

Fluctuations of temperature in the neighbourhood of the diffusion vessel would invalidate any experiment by setting up convection currents in the liquid layer. Special care was therefore taken to ensure a constant temperature. To achieve this, the experiments were carried out in a constant temperature room, maintained at the experimental temperature $\pm$ 1°C (except for the experiments at 37°C when the room temperature was maintained at 34 $\pm$ 1°C). The diffusion vessel A was further temperature controlled by being placed in a stirred water bath, the temperature of which was maintained constant within $\frac{1}{50}$ th 0°C. The measurements on olive oil were made at 25°C only. On lard, the measurements were made at four different temperatures: 21·8, 26·0, 30·5 and 37°C.

Density of the solvents

The density of the solvents were measured so that their volume inside the bottle could be determined from their weights. The density of the sample of the olive oil at 25°C was 0·921. The density of the lard was 0·915 at 25°C, and 0·904 at 35°C. The values at 25°C have been used in experiments at 21·8° and 26°C, and the other value at 35°C in the experiments at 30·5 and 37°C.

Special procedure for lard

Special procedures have to be adopted for lard during the stages of evacuation and of equilibration with the gas at the end of the diffusion period because these processes cannot be properly carried out if the lard has solidified at the experimental temperature. Once it has solidified, it does not completely melt until it is warmed up to a temperature a few degrees above 40°C.

Experiments at 26° and 22°C upon lard

The lard was melted by placing the vessel A in a water bath at 50°C. The vessel was connected to the evacuation pump through a splash bulb and evacuated for half an hour while the lard was molten. The splash bulb was then removed. The vessel A and the pump were connected to the manometer, as shown in Fig. 2. The vessel was now placed in the main water bath at 26° or 22°C; and the evacuation was continued and the lard allowed to solidify.

The lard solidified with an uneven upper surface. To make this upper surface plane the vessel A was lifted out of the water bath and plunged into a beaker of hot water to melt the upper layer of the lard, and then replaced in the bath. During this procedure, the evacuation continued through taps T_1 and T_2 of the apparatus. Half an hour was now allowed for the lard to cool to the temperature of the bath before admitting carbon dioxide.

At the end of the diffusion period, the vessel attached to the manometer was again lifted out of the bath and plunged in hot water to melt the lard now in contact with carbon dioxide. With the lard molten, the vessel was shaken for 10 to 15 minutes, and then replaced in the bath to cool, and shaking continued till the lard solidified.

Experiments at 30°C upon lard

The procedure was same as above. But at this temperature the top layer of the lard remained liquid and it was not necessary to remelt it during the stage of evacuation for obtaining a smooth upper surface.

Experiments at 37°C upon lard

The lard was melted during the preliminary stage of evacuation through the splash bulb by placing the vessel in a water bath at 50°C. Later, when the vessel was connected to the manometer and placed in the water bath at 37°C, the lard remained liquid throughout the experiment. The procedure thereafter was same as in the case of liquid olive oil.

Absolute value of the pressure readings

The manometer gives readings relative to the atmospheric pressure. The pressure readings are converted to absolute values from a knowledge of the initial barometric pressure and concurrently taken readings of a thermobarometer.

The thermobarometer shown in the diagram of

the apparatus is a Warburg manometer with an empty vessel. This manometer is charged with Brodie fluid, and set to zero at the time of taking the barometer reading. As already mentioned, the temperature of the bath is not allowed to change during an experiment. So the thermobarometer registers changes, if any, of the barometric pressure.

Calculating of the solubility and diffusion coefficient

Explanation of symbols

μ = solubility expressed as the ratio of the concentration of the gas in the liquid to its concentration in the gas phase.

K = diffusion coefficient as defined by the constant of proportionality in the Fick's equation of diffusion $\frac{dq}{dt} = - KA.\frac{dc}{dx}$. The equation states that the quantity of a substance (dq) diffusing across an area A in time (dt) is proportional to its concentration gradient ($\frac{dc}{dx}$). In c.g.s. units, K will have the dimensions cm^2/second.

The diffusion system of the present experiments is described by the following symbols:

V = The total volume of the enclosed space.

v = Volume of the liquid in the bottle

$= \frac{\text{weight of the liquid}}{\text{density}}$

L = Depth of the liquid layer = $\frac{v}{A}$, A being the cross-sectional area.

V-v = The volume of the gas phase.

P_o = Pressure of the gas at zero time, i.e. before diffusion starts.

P_{oo} = The equilibrium pressure ultimately reached between the gas and the liquid.

P_t = Pressure of the gas phase at time t.

The mass of the gas within the system remains constant during an experiment. Therefore:

$$P_o\,(V-v) = P_\infty\,(V-v) + \mu v P_\infty$$

$$\text{i.e.}\quad \mu = \frac{V-v}{v}.\frac{P_o-P_\infty}{P_\infty} \qquad \ldots\ldots\ldots\ldots(1)$$

Further, according to the work of Davidson et al (1952), the pressure of the gas phase at any time t during the early stage of the process of diffusion is given by the equation:

$$\frac{P_t}{P_o} = 1 - 2\,\frac{P_o-P_\infty}{L.P_\infty}\,\sqrt{\frac{kt}{\pi}} \qquad \ldots\ldots\ldots\ldots(2)$$

This equation can be rewritten in the form:

$$K = \frac{\pi L^2}{4t}\left(\frac{P_\infty}{P_o}\;\frac{P_o-P_t}{P_o-P_\infty}\right)^2 \qquad \ldots\ldots\ldots\ldots(2)$$

But before equations (1) and (2) can be used for calculating the values of μ and K, the values of P_o and P_∞ are to be derived from the observed data.

The value of P_o (zero time pressure)

The procedure of the experiment does not permit a direct reading of P_o. In fact, the zero time conditions of the experiment are not exactly ideal. When the gas is let in, the pressure inside the diffusion vessel does not reach a uniform value instantaneously. There is an interval of 2 seconds before the vessel is connected to the manometer. During this interval, the gas diffuses into the liquid without any resulting fall of pressure because of free communication with the source of gas supply. Then again the pressure in

the gas phase undergoes a minor readjustment when the gas in the main space and in the space inside the manometer mixes. To this list of uncertainties and sources of error about the zero time conditions, two more may be added, which have been pointed out by Davidson et al (1952). The incoming gas may have a slightly different temperature from that of the liquid in the vessel. A small quantity of the gas is absorbed by the film of oil on the exposed walls of the vessel. The zero time pressure is thus a hypothetical entity. Its value is determinable by extrapolation of the graph obtained by plotting the observed pressure readings against the square root of time. It is seen from equation (2) that this graph P_t against $\sqrt{t}$ should be linear.

Fig. 3 shows such a graph from an experiment on the diffusion of carbon dioxide in a specimen of olive oil. The earliest pressure reading of the experiment was taken at 30 seconds from the start of the stop watch, and the readings were continued up to 60 minutes. The graph has been extrapolated to its point of intersection with the vertical axis, and P_o is found to have a value of 77·75 cm.Hg. for this experiment. The slope of this linear graph $\frac{P_o - P_t}{\sqrt{t}} = 0{\cdot}152$ cm.Hg./ sec. The square of this term, namely $\frac{(P_o - P_t)^2}{t}$ enters in the calculation of K (see equation (2).

The linearity of the graph P against $\sqrt{t}$ serves

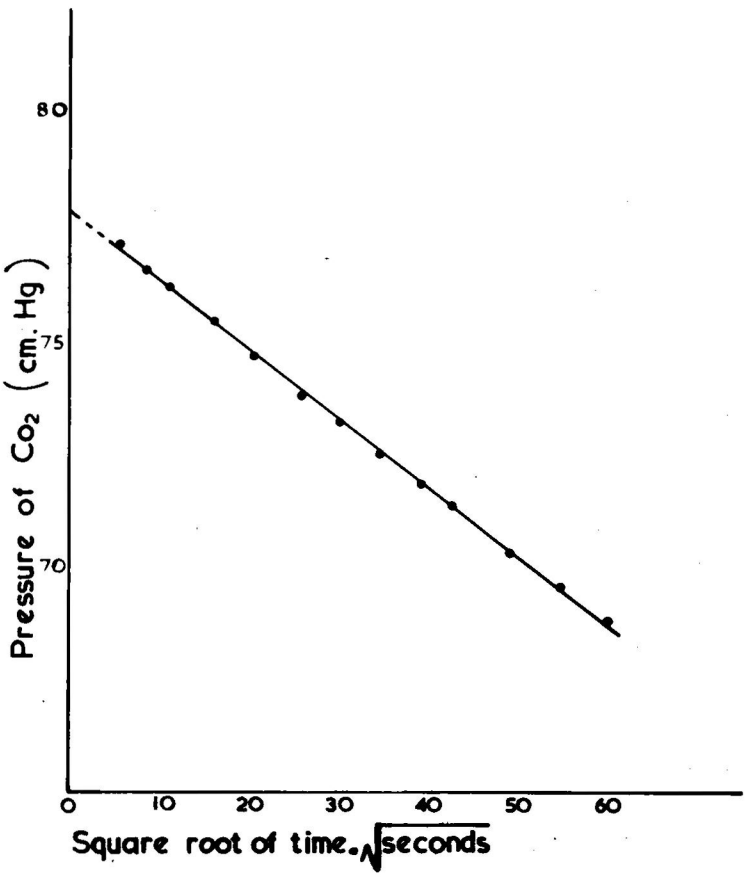

Fig. 3.

Graph showing the diffusion of carbon dioxide in a sample of olive oil. The ordinate scale gives the absolute value of the pressure in the gas phase.

the useful purpose of a criterion that the experiment has progressed as postulated by the equation (2). The graphs in Fig. 4 go to show that the diffusion of carbon dioxide in lard satisfied the above criterion. Four graphs are shown, one for each temperature of observation. The graphs have been extrapolated to zero time. The intercepts on the zero time ordinate furnish values of P_o relative to arbitrary zeros, which are different for different graphs. The absolute P_o values have been indicated at the side of the graphs. The graphs are from experiments on the same charge of lard. P_o values were of about the same order. The increase in the slope of the curves at the higher experimental temperatures signifies higher rates of diffusion at higher temperatures.

The value of P_∞ (true equilibrium pressure)

It has been mentioned before that shaking does not result in true equilibrium between the gas and the solvent. During the shaking, the gas breaks up into bubbles in which the pressure is higher than in the main gas space due to the effect of surface tension. This produces a state of slight supersaturation. Therefore, when pressure readings are taken at one and thirty minutes after shaking, the second reading is usually a little higher than the first.

The method recommended by Davidson et al (1952) for calculating the true equilibrium pressure by

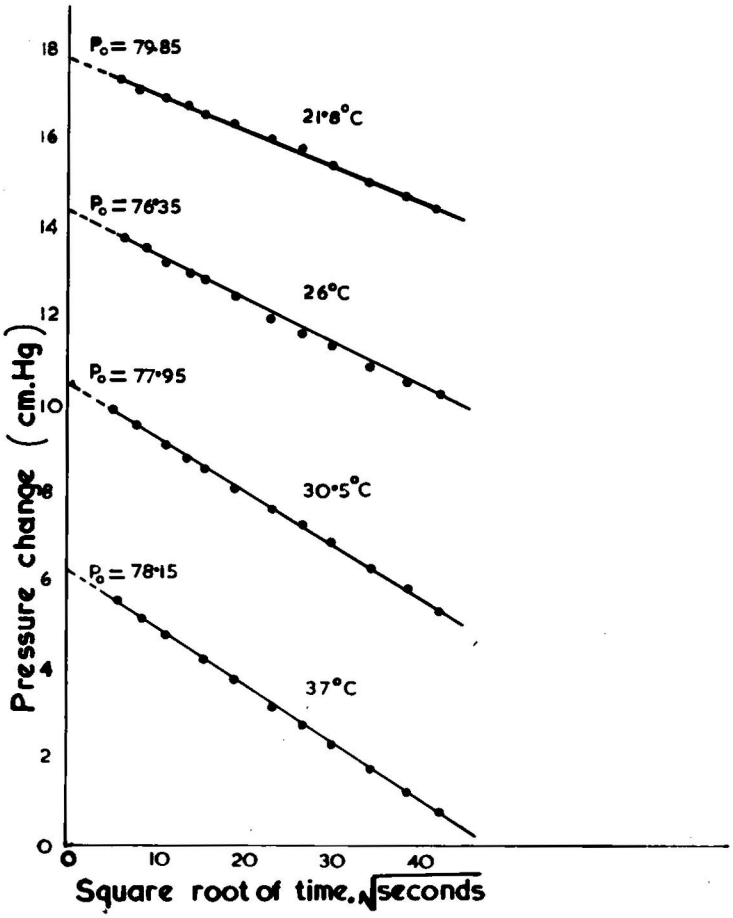

Fig. 4.

Graphs showing the decline in pressure in the closed vessel caused by the diffusion of carbon dioxide into lard at four different temperatures. The initial pressure is given against each curve; the ordinate scale is cm. of Hg.

taking into account the rates at which the pressure falls during the original inward diffusion and rises during the outward diffusion subsequent to shaking, has been adopted in the present work.

The working of the method is shown by a numerical example.

The data are from an experiment on olive oil, of which the diffusion curve is shown in Fig. 3.

P_0 = 77·75 cm.Hg.

α_1 = slope of diffusion curve = 0·152 cm.Hg./$\sqrt{\text{sec.}}$

Pressure at 1 min. after shaking = 39·48 cm.Hg.

" " 30 " " " = 39·55 cm.Hg.

α_2 = rate of rise of pressure = 0·002 cm.Hg./$\sqrt{\text{sec.}}$

Extrapolated pressure at zero time after shaking =

$$39·48 - 0·002\sqrt{60} = 39·46.$$

P_{∞} (true equilibrium pressure)

$$= 39·46 + \alpha_2 \frac{P_0 - 39·46}{\alpha_1 + \alpha_2}$$

$$= 39·46 + 0·002 \frac{77·75 - 39·46}{0·152 + 0·002}$$

$$= 39·46 + 0·50 = 40·0 \text{ cm.Hg.}$$

Estimate of error

An estimate of the magnitude of error in the values of μ and K if the above correction were not applied is of interest. An error of δP_{∞} in the value of P_{∞} introduces an error or

$$-\left[\frac{\delta P_{\infty}}{P_{\infty}} + \frac{\delta P_{\infty}}{P_0 - P_{\infty}}\right] 100\%$$

in the value of μ and an error of

$$2\left[\frac{\delta P_{oo}}{P_{oo}} + \frac{\delta P_{oo}}{P_o - P_{oo}}\right] 100\%$$

in the value of K. The above expressions of error are obtained by partial differentiation of equations (1) and (2) on page 22 . In the case of the worked out example given above the errors would amount to 2·5% and 5% respectively for the values of μ and K.

But the estimate of the correction for supersaturation depended on the observed difference between the pressure readings at one and thirty minutes after shaking. In the present investigation this difference in itself was very small, hardly more than a mm. of Hg. As the manometer was graduated in mm. the observed difference between the two pressure readings was the result of eye estimation and obviously subject to error.

The observed pressure readings at 1 and 30 minutes, and the calculated value of P_{oo} for the experiments on lard at 37°C are quoted below. In two instances there was no change in the pressure. In another instance the pressure reading at 30 minutes was a millimeter lower than at 1 minute. The pressure readings at 30 minutes were considered as the true equilibrium pressure in these instances.

Experiments on Lard. Temp. 37°C.

Experiments on Lard. Temp. 37°C.

Pressure reading (cm.Hg.) after final shaking		P_{∞}
At 1 min.	At 30 min.	cm.Hg.
54·98	54·89	54·89
54·00	54·00	54·00
54·00	54·00	54·00
47·81	47·89	48·15
47·29	47·36	47·67
50·41	50·74	51·97

Of the different quantities that enter in the calculation of μ and K, the values of P_o and P_{∞} were the quantities likely to be least accurate. The uncertainties about the value of P_o have already been mentioned. Errors in the value of P_o would influence the result almost similarly as errors in P_{∞}.

In the table of results presented later, it will be seen that the standard deviations from the mean values obtained for μ and K are fairly large. The coefficient of variation from mean values roughly amounts to 3 to 4% for μ, and 6 to 10% for K. These variations are ascribed largely to errors in the determination of P_o and P_{∞}.

Table 1

SOLUBILITY AND DIFFUSION OF CO_2

Solvent - Olive Oil

Temperature	No. of Experiments	Mean K $x10^6 cm^2/sec.$	S.D. $x10^6$	Mean μ	S.D.
25°C	5	4·35	± 0·07	1·34	± 0·03

Solvent - Lard

Temperature °C	No. of Experiments	Mean K $x10^6 cm^2/sec.$	S.D. $x10^6$	Mean μ	S.D.
21·8	5	3·09	± 0·37	1·13	± 0·03
26·0	4	5·19	± 0·28	1·14	± 0·07
30·5	5	6·27	± 0·30	1·18	± 0·03
37·0	6	7·34	± 0·72	1·14	± 0·04

---oOo---

RESULTS

The results of the measurements are presented in Table 1. This table shows that the solubility of carbon dioxide in lard remains unchanged in spite of gross changes in the physical state of the lard. At 37°C the lard is liquid; at 26°C it is apparently solid, a thick suspension of microcrystalline material in an oily matrix.

The diffusion coefficient is low at the lower temperatures but the reduction is not as great as the change in the state of the solvent.

It has also been found by Eggleton, Elsden, Feglar and Hebb (1945) and by Davidson et al (1952) that the solubility and diffusion coefficient of the gases studied by them (H_2, N_2, and O_2) did not differ markedly between olive oil and solidified lard.

Two explanations of this observed similarity of the behaviour of gases in the solid and liquid states of a fat are possible. Either the gases can dissolve in and diffuse through crystals of the solid lard, or as the lard solidifies the liquid matrix becomes supersaturated due to extrusion of dissolved gases from the material which has become crystalline. In the determination of the solubility the end equilibrium pressure between the lard and the dissolved gas was obtained by heating the lard to liquefaction and then allowing it to cool to the temperature of the experiment. To avoid

the risk of supersaturation, the procedure was to take two readings of the final pressure with an interval of half an hour between them. The two readings did not/differ by more than 1 mm. of mercury in the present experiments on lard.

usually

It is, however, possible that half an hour was too small an interval of time to detect any rise of pressure which might be an extremely slow process. To examine this possibility, the following experiment was performed:

A glass bulb of accurately measured capacity charged with a known volume of lard was fused to the side arm of an inverted L shaped tube, the height of which was 85 cm. with the open end dipping into a reservoir of mercury. Initially there was an opening at the bend of the tube, through which the system was thoroughly evacuated. Pure dry carbon dioxide was let in and the opening sealed off. During the evacuation, the lard was melted and allowed to solidify. After introduction of carbon dioxide, it was melted again and shaken. A quantity of carbon dioxide was absorbed by the lard and the level of mercury in the vertical arm of the tube rose to a certain height. This arm now acted as a manometer and indicated the pressure of the gas phase.

The whole set-up was left at room temperature. From time to time the height of the mercury column in the tube, the room temperature and the barometric

pressure were recorded. The mass of lard in the bulb, the capacity of the bulb and the bore of the glass tube were known. With these data, the value of $\frac{PV}{T}$ in respect of the gas phase was followed for several months and was found to remain constant at $5{\cdot}10 \pm 0{\cdot}05$. This shows that carbon dioxide dissolved in liquid lard is not extruded by allowing the lard to solidify and to remain so for months. But whether the gas has been retained within the crystals of fat or outside in the liquid matrix, which remain indefinitely supersaturated, one cannot say.

Davidson et al (1952) have attempted to decide whether carbon dioxide can dissolve in crystals of fatty acid esters such as ethyl palmitate and stearate. The main difficulty of a direct approach to the problem was to be satisfied that the ester had completely crystallized before carbon dioxide was introduced; carbon dioxide being highly soluble in the liquid esters, the presence of a small amount of liquid amongst the crystals would lead to erroneous results.

They found that ethyl palmitate evacuated in its liquid state and allowed to crystallize in vacuo for over 4 hours did not dissolve nitrogen gas. They considered this as a satisfactory test of complete crystallization of the ester. The nitrogen gas was then pumped out and carbon dioxide admitted. There was an appreciable fall of pressure, and a

straight line was obtained when the pressure readings were plotted against square root of time. On this observation they based their conclusion that carbon dioxide could dissolve in, and diffuse through, the crystals of ethyl palmitate, whereas nitrogen did not.

Fig. 5 shows the result of a series of three experiments of the type described above, carried out by the present observer on a sample of ethyl palmitate.

An attempt was made to purify commercial ethyl palmitate by fractional distillation under reduced pressure; and finally by fractional crystallization. Several fractions were obtained which crystallized at different temperatures, ranging from 30° to 15°C. An appreciable fraction remained liquid even at 15°C. None of the samples showed a really sharp melting or solidifying point but were found to melt or solidify within comparatively narrower ranges of temperature ($\pm$ 0·5°C).

The graphs shown in Fig. 5 were obtained from experiments on a sample of melting point of 25° $\pm$ 0·5°C. The sample crystallized at 23°C. The three experiments illustrated were all on the same charge of the ester and at the same experimental temperature. The graphs have been arranged in the order of their slope. Individually considered, the graphs lend support to the conclusion of Davidson et al (1952) that carbon dioxide was diffusing

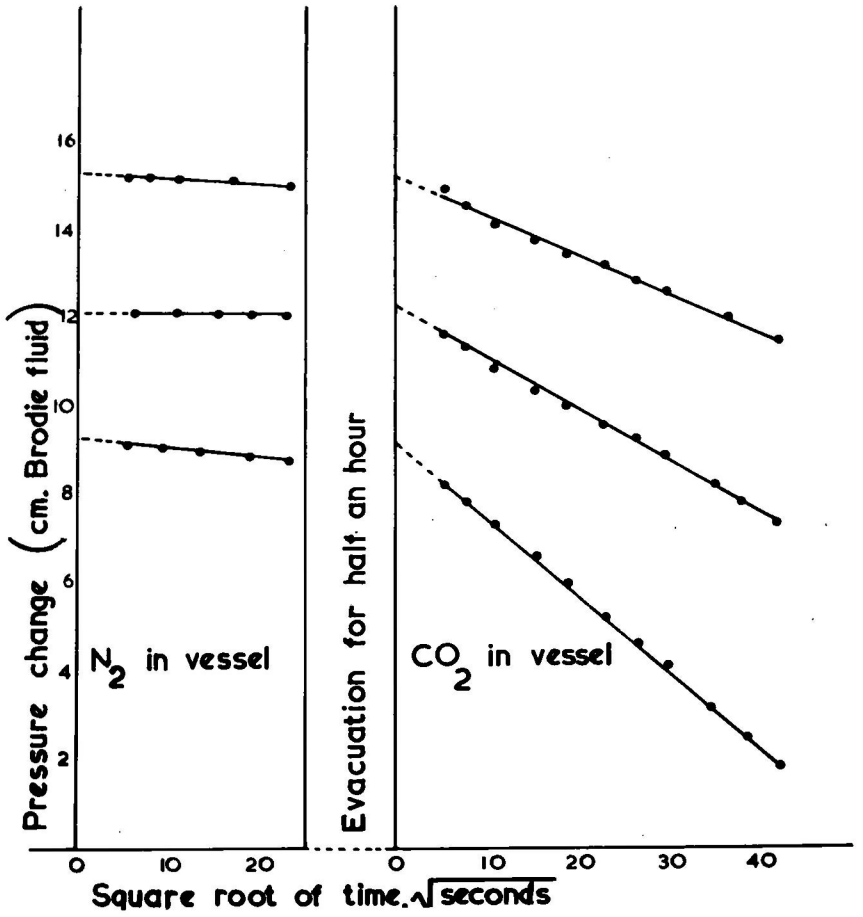

Fig. 5.

Graphs showing a comparison of the behaviour of nitrogen and carbon dioxide in crystalline ethyl palmitate. The initial pressures of the gases in the experiments were about 1 atmosphere (1000 cm. of Brodie's fluid).

into crystals into which nitrogen could not diffuse. But when considered together they make it difficult to explain why the slopes should differ from observation to observation if crystallization was complete.

A closer inspection of the curves representing the behaviour of nitrogen shows that the initial pressure of the gas was not invariably maintained; one of the curves shows a fall of about 4 mm. of Brodie fluid. The graph in the paper of Davidson et al (1952) is also suggestive of a slight fall in the pressure of nitrogen. Carbon dioxide has been found to be 17 times more soluble than nitrogen in liquid ethyl palmitate. Since both gases have about the same rate of diffusion in liquid ethyl palmitate, the fall of pressure of nitrogen in a given time should be about 17 times smaller. (The solubility and diffusion of carbon dioxide in liquid ethyl palmitate were measured separately during the present research.)

A fall of a few mm. of Brodie fluid in the pressure of nitrogen, which may be considered as insignificant, will be magnified to a few cm. when nitrogen is replaced by carbon dioxide. Therefore, since the behaviour of the nitrogen does not, with certainty, exclude the possibility of the presence of liquid palmitate, the results shown in Fig. 5 cannot be considered as really convincing evidence of the solubility of carbon dioxide in crystalline esters.

How carbon dioxide has apparently the same

solubility in liquid and solid lard, the present investigations fail to explain.

DISCUSSION

The values of solubility shown in the table of results are Ostwald's absorption coefficient being the ratio of the concentration of the gas in the liquid to its concentration in the gas phase at equilibrium. If these values are multiplied by $\frac{273}{273 + t}$, they are converted to Bunsen's absorption coefficient, i.e. c.c. of carbon dioxide (0°C and 760 mm.Hg.) which at the temperature of the experiment is dissolved in 1 c.c. of the solvent under a pressure of one atmosphere.

The values of Bunsen's absorption coefficient of carbon dioxide in olive oil and lard calculated from the results of the present experiment are:

1·23 c.c. CO_2 (0°C 760 mm.) per c.c. olive oil (25°C)

0·98 c.c. CO_2 (0°C 760 mm.) per c.c. lard (37°C)

1·06 c.c. CO_2 (0°C 760 mm.) per c.c. lard (30°C)

1·04 c.c. CO_2 (0°C 760 mm.) per c.c. lard (26°C)

1.05 c.c. CO_2 (0°C 760 mm.) per c.c. lard (22°C)

These values are comparable to the values obtained by Vibrans (1935) and Schaffer and Haller (1943).

1.34 c.c. CO_2 (0°C 760 mm.)/c.c. cotton seed oil (22°C) (Vibrans, 1935)

1·02 c.c. CO_2 (0°C 760 mm.)/c.c. lard (45°C) (Vibrans, 1935)

1·00 c.c. CO_2 (0°C 760 mm.)/c.c. lard (40°C) (Schaffer and Haller, 1943)

The absorption coefficient of carbon dioxide in blood serum at 38°C is 0·510 c.c./c.c. of serum (Van Slyke, Sendroy, Hastings and Neil, 1928). The partition coefficient of carbon dioxide between fat and blood serum is thus nearly 2.

In the body of an average man described by Keys and Brozek (1953) fat amounts to 14% of the body weight. A 70 kilo man has about 11 litres of fat in his body (density of fat = 0·9 g./c.c.). Since fat can hold carbon dioxide only in a physically dissolved state, the total storage capacity of the body fats for carbon dioxide hardly exceeds 750 c.c., (assuming for it a partial pressure of 50 mm.Hg. in fatty tissues). In the very obese in whom the total body fats may amount to 60 litres (54 kilos; McCance and Widdowson, 1951) the quantity of carbon dioxide that would be present in the fat phase under the above partial pressure would be about 4 litres.

The diffusion coefficients determined in the present work are Fick's coefficients and have been expressed in units of cm^2/sec. At 37°C carbon dioxide is found to have a diffusion coefficient of $7·34 \times 10^{-6}$ cm^2/sec. which is same as $4·40 \times 10^{-4}$ cm^2/min.

If this value of $4·4 \times 10^{-4}$ cm^2/min. is multiplied by 0·98, the value of the Bunsen's absorption coefficient of carbon dioxide in lard, the result, $4·3 \times 10^{-4}$, represents the number of c.c.

of carbon dioxide (0°C 760 mm.) which would in one minute pass through an area of one sq.cm. in lard, under a pressure gradient of one atmosphere per cm. In other words, $4{\cdot}3 \times 10^{-4}$ c.c./cm^2/min. is the value of the permeability constant of carbon dioxide in lard at 37°C under a steady pressure gradient of 1 atmosphere/cm.

It may be mentioned here that what Krogh (1919) defined to be the diffusion constant in his work was really permeability constant. His estimate that carbon dioxide has a diffusion constant of 4 (Krogh units) in the connective tissue of frog, means that 4 c.c. of CO_2 would pass through one sq.cm. of the tissue per minute if a steady pressure gradient of one atmosphere per micron of thickness were maintained. When the pressure gradient considered is one atmosphere per cm. instead of per micron of thickness, then the value of Krogh's diffusion constant of carbon dioxide in the connective tissue becomes 4×10^{-4} c.c./cm^2/min., a value similar to what has been found now as its value in lard at 37°C. The measurement by Krogh was made on a piece of connective tissue obtained from the abdominal musculature of frog after removal of the muscle fibres, and was performed at 20°C.

To facilitate further comparisons of the present data with other reported data on the diffusion of carbon dioxide in different media, a table compiled by Wright (1934) is reproduced

THE DIFFUSION OF CARBON DIOXIDE IN VARIOUS MEDIA

(Reproduced from Wright, 1934)

Media	Temperature	Permeability constant	Absorption coefficient (Bunsen's)	Diffusion coefficient	
	°C.	x 10^4	c.c./c.c.	cm^2/min. x 10^4	
Water	16	9·4	0·99	9·5	Hüfner, 1897
Rubber	17	0·44	0·86	0·51	Daynes, 1920
Rubber	22	0·48	0·93	0·51	Wright, 1934
Gelatin 20 per cent	15	5·9	1·0	5·9	Hagenbach, 1898
Connective tissue (frog)	20	4·0	0·73	5·5	Krogh, 1919
Connective tissue (dog)	22	2·7	0·73	3·7	Wright, 1934
Muscle (frog)	22	0·85	0·78	1·17	Fenn, 1928
Muscle (frog)	22	5·3	0·78	6·8	Wright, 1934
Muscle (dog)	22	4·7	0·78	6·0	Wright, 1934
Smooth muscle (cat)	22	5·0	0·78	6·4	Wright, 1934
Nerve (frog)	22	0·55	0·78	0·71	Fenn, 1928
Frog (skin)	22	3·1	0·73	4·2	Wright, 1934
Calculated from the present work					
Olive oil	25	3·2	1·23	2·61	
Lard	37	4·3	0·98	4·4	
"	30	4·0	1·06	3·76	
"	26	3·28	1·04	3·11	
"	22	1·9	1·05	1·85	

Permeability is expressed in c.c. per cm^2 per min. under a pressure gradient of one atmosphere per cm x 10^4.

The coefficients of diffusion determined in the present work have been multiplied by 60 to change the unit of time to minutes. The coefficients of solubility have been converted to Bunsen's coefficients. Permeability constants have been calculated by multiplying the coefficients of diffusion with Bunsen's coefficients.

(Table 2) together with the present data expressed in the same manner as other data in the table. An examination of this table shows that the rate of diffusion of carbon dioxide in pure fat at body temperature is of the same order of magnitude as its rate of diffusion in the connective tissue and skin, but is somewhat lower than its rate in the muscle tissue. Fenn's (1928) values for the diffusion coefficient of carbon dioxide in frog muscle and nerve are, however, lower. Wright (1934) has pointed out that Fenn's measurements were of an approximate nature and no account was taken of the formation of bicarbonate during the process of diffusion.

The object of measuring the rate of diffusion of carbon dioxide in animal fat was to find out if its rate of diffusion was likely to have any limiting influence on its distribution in fat rich tissues of poor blood supply.

Gersh and Still (1945) have recently studied the distribution of blood capillaries in the adipose tissue of rats. According to them, the ratio of the surface of the capillary bed to the volume of tissue supplied by the vessels ($\frac{\text{Surface}}{\text{Volume}}$ ratio) is 52 cm.$^{-1}$ in the fat rich tissue of the rat. During ordinary activity, about one half of the capillaries are open ($\frac{\text{Surface}}{\text{Volume}}$ = 24 cm.$^{-1}$).

As an example, an extremely artificial case may be considered. A flat sheet of fat of thickness 400μ exposed at one surface to blood has a

$\frac{\text{Surface}}{\text{Volume}}$ ratio of 25 cm.$^{-1}$. If a sheet of fat of the above dimension is suddenly exposed to carbon dioxide, its percentage saturation at any time thereafter can be calculated by the following equation:

$$\frac{Q}{Q_1} = 1 - \frac{8}{\pi^2}\left[e^{-\frac{\pi^2}{4}\frac{kt}{a^2}} + \frac{1}{9}e^{-\frac{9\pi^2}{4}\frac{kt}{a^2}} + \frac{1}{25}e^{-\frac{25\pi^2}{4}\frac{kt}{a^2}} + \right]$$

where

Q_1 = the total amount of gas absorbed at saturation.

Q = amount absorbed in time t.

a = thickness of the sheet.

K = Fick's diffusion coefficient (Andrews and Johnston, 1924).

Neglecting all terms of the series except the first, the equation can be reduced to a simpler form sufficiently accurate for the present purpose.

$\frac{Kt}{a^2} = -0{\cdot}0851 - 0{\cdot}933 \log\left(1 - \frac{Q}{Q_1}\right)$ (Wright, 1934)

In the example under consideration

$K = 7{\cdot}3 \times 10^{-6}\text{cm}^2/\text{sec.}$

$a = 400 \times 10^{-4}$ cm.

The time required for 50% saturation is, therefore, = 43 seconds.

The above example is highly artificial but it does indicate that the rate of diffusion of carbon dioxide in fat will not be a cause of delay in the establishment of an equilibrium between fatty tissues and blood beyond/about two minutes, following a change in the partial pressure of carbon dioxide in the blood. Perhaps the time scale would be much

smaller than has been implied by the over simplified calculation. In the actual tissue the capillaries run in all direction. If it is assumed that the capillaries are all parallel to one another and have an average diameter of 6μ, the intercapillary distance corresponding to a $\frac{\text{Surface}}{\text{Volume}}$ ratio of 25 cm.$^{-1}$ will be about 90μ. The diffusive distance to the nearest blood capillary would be of the order of 50μ and not 400μ as considered in the extreme example. As a matter of fact, Gersh and Still (1945) have found in their work that practically every fat cell is in contact with a capillary at some point of its surface, and the fat cells have an average dimension of 100μ x 60μ in the fat rich tissue.

On this topic of diffusion an interesting comparison can be made between experiments of Eggleton, Elsden, Fegler and Hebb (1945) on the rate of elimination of nitrogen from the lungs of dogs exposed abruptly to an atmosphere of pure oxygen, and experiments of Shaw and Messer (1930) on the rate of desaturation of carbon dioxide from cats equilibrated previously to a higher than normal partial pressure of alveolar carbon dioxide (by artificial ventilation with carbon dioxide enriched air).

Eggleton et al (1945) found that the average nitrogen elimination curve of their five dogs, when corrected for the 'skin leakage', was practically

linear with respect to the square root of the time of exposure to oxygen for the first half of the process.

A linear relationship between the amount of a substance given away and the square root of time elapsed is characteristic of diffusion phenomenon (Hill, 1928; Eggleton, Eggleton and Hill, 1928). On this ground it has been suggested by Eggleton (1952) and Davidson et al (1952) that the speed of elimination of nitrogen is probably limited by its speed of diffusion from the cell in which it is dissolved to the nearest blood capillary.

Consider now the experiments of Shaw and Messer (1930). Cats under anaesthesia were artificially ventilated with air containing 11% carbon dioxide for an hour and a half, and were considered to have come to an equilibrium with the raised partial pressure of carbon dioxide in the alveoli of the lungs, which was about 50 mm. of mercury above normal. At the end of this saturation period, the inspiratory end of the respiratory valve was connected to outdoor air and the expiratory end to a three-way tap through which the expired air was collected continuously in one of two spirometers in turn. The carbon dioxide discharged in the expired air in excess of that produced by metabolism during the collection period represented that which had been retained by the body as a result of the previous saturation to a

higher carbon dioxide level. The per cent desaturation, which is attained at any given time, has been calculated as the ratio which the extra carbon dioxide eliminated during that time bears to the total carbon dioxide retained.

The desaturation curve for carbon dioxide obtained by these investigators is shown by the upper graph in Fig. 6. The curve represents the average of ten experiments. In this graph the state of desaturation expressed as a percentage has been plotted against time in minutes.

The lower graph has been constructed by the present worker on the same data by plotting the percentage of desaturation against square root of time in seconds.

It is interesting to observe that, when thus redrawn against square root of time, the desaturation of carbon dioxide curve of Shaw and Messer (1930) becomes linear till the process is two-thirds complete. What is more striking is that on extrapolation the graph passes through the origin. The slope of the linear part of the graph is 1·5 per cent per $\sqrt{\text{second}}$. A value of 2 per cent per $\sqrt{\text{second}}$ has been considered by Davidson et al (1952) to be the slope of the nitrogen elimination curve of dogs exposed abruptly to an atmosphere of oxygen and they have regarded the nitrogen elimination curve of the animal to be a 'diffusion curve'. One wonders whether the same argument will apply to the

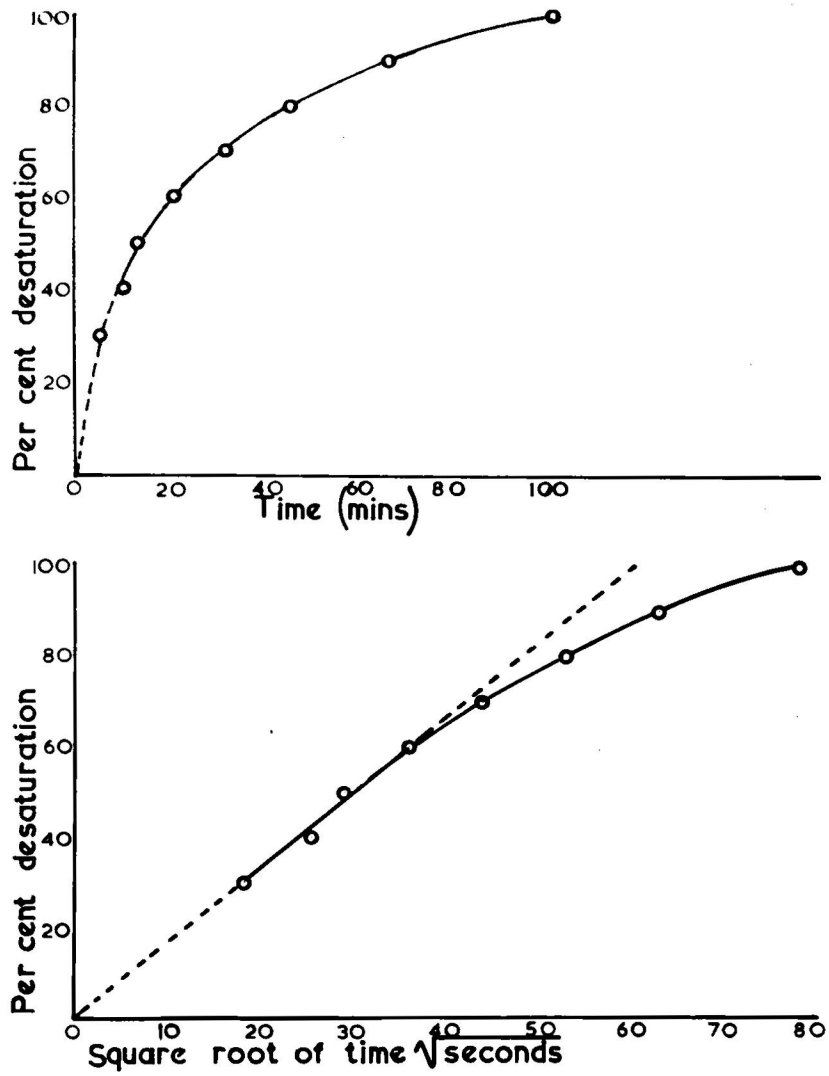

Fig. 6.

Upper curve: Carbon dioxide desaturation curve of cats based on the data of Shaw and Messer (1930).

Lower curve: The same data plotted against square root of time.

carbon dioxide desaturation curve and whether it could also be regarded as a 'diffusion curve'.

The experiments of Shaw and Messer (1930) indicate that it takes a long time, about 100 minutes according to their estimate, for the body of a cat to come into equilibrium with an altered partial pressure of carbon dioxide in the alveolar air. Their estimate as regards the magnitude of extra carbon dioxide retained or given out by the body when it comes into equilibrium with an altered pressure of carbon dioxide was 1·8 c.c. per kilo body weight per mm. of mercury pressure difference. According to this estimate, a rise of 50 mm. of mercury in the alveolar pressure of carbon dioxide will cause a retention of 90 c.c. of carbon dioxide within the body. They found that the carbon dioxide capacity of a kg. of cat's blood was 3·2 c.c. CO_2/mm.Hg. pressure difference. They considered that a kg. of mixed tissue of cat contained 55 c.c. blood and 645 c.c. of tissue fluid. Assuming that the whole of the retained carbon dioxide would be held between the blood and tissue fluid, they calculated the carbon dioxide capacity of a kg. tissue fluid exclusive of blood to be 2·49 c.c. CO_2/mm.Hg. pressure difference. This means that if 90 c.c. carbon dioxide is retained 10 c.c. will be held in the blood and 80 c.c. in the tissue fluids.

It will be observed that they did not make a

distinction between the extracellular and intracellular fluids. Intracellular fluid has about one-third the carbon dioxide capacity of extracellular fluid (Wallace and Hastings, 1942). Taking into account this difference between the extra and intracellular fluids and assuming that the cat's body has a composition similar to the composition of human body, shown in Fig. 1 of the present work, the aqueous fraction of a kg. mixed tissue of cat is equivalent to 380 c.c. of a fluid of the same carbon dioxide capacity as extracellular fluid or blood. For a rise of 50 mm.Hg. in carbon dioxide pressure, 380 c.c. of blood will be able to accommodate $380 \times 50 \times 3{\cdot}2 \times \frac{1}{1000} = 60$ c.c. of carbon dioxide only. Thus of the 90 c.c. of extra carbon dioxide supposed to be retained per kg. of body weight of a cat, the aqueous phase of the body can account for only 60 c.c.

Where will the remaining 30 c.c. be distributed? If the fat in cat's body amounts to 14% body weight, then from the results of the present measurement on the solubility of carbon dioxide in fat it can be calculated that 10 c.c. of the retained carbon dioxide will find its way into the body fat. Even then 20 c.c. would remain unaccounted for.

The magnitude of the quantity of carbon dioxide retained in the body of an animal forced to breathe air containing a very high percentage of carbon dioxide and not accounted for by the amount

retained in the soft tissues, has been found to be very much greater, by Irving, Ferguson and Plewes (1930) and Freeman and Fenn (1953), than the above calculation, based on the observations of Shaw and Messer, indicate. The duration of the experiments of Freeman and Fenn was from a few days to a few weeks,and of Irving et al (1930) 3 hours.

The data of these experiments strongly suggesting that the bone mineral of the animal is an important site of retention of carbon dioxide will be discussed in the next section of the present work dealing with the question of carbonate exchange between bone and surrounding fluid.

The investigations on the solubility and diffusion of carbon dioxide in fat carried out in this part of the work indicate that the state and distribution of carbon dioxide in body fats do not explain the length of time required by the animal body to come into equilibrium with an altered pressure of carbon dioxide in the lungs, and can account for only a small fraction of the amount of carbon dioxide involved in the change.

SUMMARY AND CONCLUSIONS

The coefficient of diffusion and the solubility of carbon dioxide have been measured in olive oil and in lard.

The measurements on lard were made at four different temperatures, 22°, 26°, 30° and 37°C. The state of the solvent was liquid at 37°C and apparently solid at 22° and 26°C; but the solubility of carbon dioxide in the solid and liquid state of the solvent was not found to be significantly different. The coefficient of solubility was very nearly 1·1 at all the temperatures.

The diffusion coefficient of carbon dioxide in lard (liquid) at 37°C was $7{\cdot}3 \times 10^{-6} cm^2/sec$; at 22°C when the lard was apparently solid it was $3{\cdot}1 \times 10^{-6} cm^2/sec$. The rate of diffusion of carbon dioxide in solid fat did not appear to be materially low.

The data of the present investigation have been compared with other reported data in the literature on the diffusion of carbon dioxide in different media. The rate of diffusion of carbon dioxide in fat at body temperature is of the same order of magnitude as its rate of diffusion in connective tissue and skin, but is somewhat lower than its rate of diffusion in muscle tissue.

It is calculated that the rate of diffusion of carbon dioxide in fat is not likely to delay the

establishment of equilibrium between blood and fat-rich tissues of very poor blood supply, beyond a minute or two, following a change in the partial pressure of carbon dioxide in the blood.

The state and distribution of carbon dioxide in body fats do not explain the length of time required by the animal body to come into equilibrium with an altered pressure of carbon dioxide in the lungs, and can account for only a small fraction of the amount of carbon dioxide involved in the change.

PART II

EXCHANGEABILITY OF CARBONATE WITH PHOSPHATE IN BONES

INTRODUCTION

Phosphate and carbonate are the two chief anions in the inorganic fraction of bones. The object of the present investigation was to determine how far these two anions were exchangeable when intact bones were placed in solutions widely different in phosphate and carbonate concentration.

M.G. Eggleton (1933) observed that frog bones absorbed considerable quantities of phosphate from surrounding Ringer solutions. The solutions employed in these experiments contained inorganic phosphate as well as calcium ions, and the process of phosphate deposition was considered as a phenomenon similar to the 'inorganic mechanism' of bone salt deposition described by Robinson, Macleod and Rosenheim (1930).

This 'inorganic mechanism' referred to the deposition of bone phosphate in slices of rachitic cartilage placed in solutions containing calcium and inorganic phosphate. It was different from the 'phosphatase mechanism' acting on a substrate of organic phosphate.

The presumption, therefore, in the experiments of Eggleton (1933) was that the disappearance of the phosphate ions was due to their deposition in the form of calcium phosphate. This might have been so. But in view of the fact that the work of Robinson et al (1930) was on rachitic cartilage and not actually on bones and further as some of the

experiments of Eggleton (1933) showed transfer of phosphate from the bones to the surrounding medium at a pH of 8, at which pH calcium phosphate is unlikely to dissolve, an explanation of the phenomenon in terms of reversible solution and deposition of calcium phosphate becomes rather difficult.

An alternative explanation of the experiments of Eggleton (1933) is that the phosphate ions of the surrounding solution replaced carbonates in the bone. If this were so, it should be possible to demonstrate exchange of phosphate between the surrounding medium and the bones in the absence of calcium in the surrounding solution.

Accordingly, in the first group of experiments of the present work, the bones have been placed in isotonic salines containing small amounts of either phosphate or bicarbonate but no calcium, and the change in the phosphate concentration of the surrounding medium studied to see if the phenomenon of phosphate exchange observed by Eggleton (1933) occurred in the absence of calcium. In the second group of experiments the bones were placed in isotonic phosphate or bicarbonate solutions for different lengths of time and the bones then analysed to find the effect of such procedure on their phosphate and carbonate content.

METHODS

Preparation of the Saline Media

Three stock isotonic solutions of the following compositions were prepared:-

1. Isotonic sodium chloride

Sodium chloride	0·7 g.
Water to	100 ml.

2. Isotonic phosphate

Na_2HPO_4	0·925 g.
$NaH_2PO_4 2H_2O$	0·339 g.
Water to	100 ml.

3. Isotonic bicarbonate

$NaHCO_3$	1·004 g.
Water to	100 ml.

The molar ratio of disodium to monosodium phosphate in the isotonic phosphate solution was 3 to 1 which fixed its pH at very nearly 7·3. At the same time the total osmolar concentration of sodium and phosphate ions in it equalled 239·1 milli os mol per litre, making it isotonic with 0·7% NaCl.

Three ml. of this stock phosphate solution made up to 100 ml. with isotonic sodium chloride gave an isotonic solution of pH about 7·3 and containing approximately 80µg P per ml. This was the phosphate saline used in the first group of experiments. The corresponding bicarbonate saline was prepared by mixing 3 ml. of the isotonic bicarbonate with 97 ml. of isotonic sodium chloride. The bicarbonate saline thus prepared was slightly more

alkaline than the phosphate saline. It was found that by adding a drop of $\frac{N}{10}$ HCl to 3 ml. of the bicarbonate saline and immediately putting a stopper on the test tube, the requisite adjustment in pH could be made so as to give the same tint of greenish yellow with universal indicator (B.D.H.) as the phosphate saline.

For some experiments of the first group the isotonic sodium chloride was shaken to saturation with a quantity of chloroform before using it to dilute the phosphate and bicarbonate solutions. It was considered desirable to see the effect of chloroform on the process because Robinson et al (1930) had found chloroform to be inhibitory to the 'inorganic mechanism' of bone salt deposition.

For the second group of experiments the isotonic phosphate and bicarbonate solutions were used without any dilution with sodium chloride solution, the object being to induce maximum change in the bones.

The bicarbonate solution required the addition of 0·3 ml. $\frac{N}{10}$ HCl per 5 ml. for adjustment of its pH to that of the phosphate solution.

The Bone Preparation

The experiments were performed with frog bones. The femur and tibiofibula of freshly killed frogs were dissected out and cleaned free of soft tissues as far as practicable. The cleaned bones were washed by leaving them in isotonic sodium chloride

solution for half an hour. Before being placed in the test solutions, the bones were wiped with moist filter papers and weighed. In interpreting the results of experiment, it was found necessary to have an idea of the volume of the bones, so in a number of cases the specific gravity of the combination, femur and tibiofibula, were determined. For this purpose the bones were weighed inside a specific gravity bottle. The bottle was next filled up with isotonic saline, first with the bones inside and then without the bones, and weighed. The average specific gravity was 1·24 (average of 8 determinations).

EXPERIMENTS OF GROUP 1

The femur and tibiofibula of one side, prepared as described above, were transferred into a test tube containing 3 ml. of phosphate saline. A similar test tube with 3 ml. of the same phosphate saline was set up as a control, analysis of which gave the initial concentration of phosphate in the medium surrounding the bone. The corresponding bones of the opposite side were placed in 3 ml. of bicarbonate saline, a drop of 0·1N HCl added and the test tube tightly stoppered to prevent escape of carbon dioxide. In the control test tube only a drop of 0·1N HCl was added to 3 ml. of the bicarbonate saline and test tube kept stoppered.

The test tubes were placed inside a refrigerator overnight. After a period of 17

hours, 1 ml. aliquot portions of the saline media were removed from the test tubes and their phosphate content determined by the method of Fiske and Subbarow (1925). The photometric measurements were done with a Pulfrich Visual Photometer, using 720mμ filter.

Eight experiments of this type were performed, in 4 of which the saline media was saturated with chloroform.

EXPERIMENTS OF GROUP 2

In this group of experiments, bones of one side were placed in isotonic phosphate and bones of the opposite limb in isotonic bicarbonate solution for 4, 12 or 24 hours. At the end of the specified period the bones were lifted out of the solutions, washed quickly with distilled water and their total carbon dioxide and inorganic phosphate content determined in the manner described below. To obtaine zero hour values, in a number of experiments the bones were removed from the phosphate and bicarbonate solutions immediately on being immersed in them.

Heat treatment : In 3 experiments the bone preparations were immersed in boiling normal saline for 30 seconds immediately before placing them in the test solutions.

Determination of Bone Carbonate

The bones were introduced into a cylindrical glass vessel containing 10 ml. of N-HCl, over

boiling water bath. The vessel had a ground glass stopper with inlet and outlet tubes. A gentle stream of carbon dioxide free air was drawn through this acid digestion chamber. The carbon dioxide expelled from the bones and escaping in the air-stream was absorbed in two other vessels placed in series containing accurately measured 10 ml. volumes of $\frac{N}{10}$ NaOH. By regulating the air stream to a rate of about 50 to 100 bubbles per minute and placing a coiled length of glass tubing in its path through the alkali trap, over 95% of the carbon dioxide was absorbed in the first trap and the rest in the second. The risk of carbon dioxide escaping unabsorbed was negligible and less than 0·25%. Twenty minutes of digestion was sufficient for the complete destruction of the bones. The quantity of carbon dioxide absorbed by the alkali traps was determined by Van Slyke and Neil (1924) manometric method.

The reliability of the method as tested by estimating the carbon dioxide content of crystals of calcite of known weight, is shown in the table below.

Estimation of CO_2 in Calcite

Vol. of CO_2 expected from weight of calcite c.c. at 0°C. and 760 mm.Hg.	Vol. of CO_2 experimentally measured c.c. at 0°C. and 760 mm.Hg.
2·35	2·34
2·98	2·88
3·10	3·05
2·72	2·70

In the method described by Sobel, Rockenmacher and Kramer (1944) the whole of the carbon dioxide expelled from the bones during acid digestion is directly absorbed by alkali inside the extraction chamber of a Van Slyke's apparatus and then estimated. The method, therefore, required an accessory fitting to the usual apparatus. Moreover, if the total volume of carbon dioxide evolved from the bone is expected to greatly exceed 1 c.c. (as it did in most experiments of the present investigation), then a chamber with a graduation at 5 c.c. level has to be employed. In the method adopted in the present investigation the carbon dioxide was absorbed separately and, as only a known fraction of the alkali used in absorbing the carbon dioxide was introduced into the Van Slyke's apparatus, all measurements could be made at 2 c.c. level of the usual apparatus and no accessory fitting was needed.

Estimation of Phosphate in the Acid Digest of Bones

The acid digest of the bones after expulsion of carbon dioxide was quantitatively transferred to a volumetric flask and made up to 100 ml. with distilled water. In trying to estimate phosphate in this solution there appeared a floculent precipitate on addition of molybdate reagent. This complication has been reported before by Shear and Kramer (1928). To overcome the difficulty they pretreated the bone digest with molybdate and filtered off the precipitate before addition of the

colour developing reagent. The same procedure was adopted in the present work but centrifuging was preferred to filtering off the precipitate. Detailed procedure was as follows: 5 ml. of the diluted bone digest was transferred to a 50 ml. volumetric flask, 1 ml. molybdate II reagent (Fiske and Subbarow, 1925) was added and the volume made up to 50 ml. with distilled water. After allowing to stand for 15 minutes, 20 ml. of the solution was centrifuged for 10 minutes at 3500 r.p.m. Measured volumes of the clear supernatant fluid was pipetted into 10 c.c. volumetric flasks and phosphate estimation done by Fiske and Subbarow's method. Shear and Kramer (1928) have recorded that the separation of the molybdate precipitate does not remove any phosphate with it. The possibility of such loss was further investigated in the present work by finding out whether extra phosphate added to a bone digest could be correctly determined by the method. The result obtained in a number of experiments of this type is shown below:

Amount of P added	Amount found by measurement
160 µg	168 µg
160 µg	168 µg
160 µg	154 µg
40 µg	39 µg
40 µg	39 µg

It became clear that the phosphate values of bones determined by the above method would have

considerable margin of error but it was considered that the error was not large enough to vitiate the conclusions drawn from a number of experiments.

RESULTS

EXPERIMENTS OF GROUP 1

The results of the experiments is shown in Table 3. A comparison of the figures in columns 3 and 4 and columns 6 and 7 shows that in all of the experiments, including those in which the saline was treated with chloroform, there was loss of phosphate from the phosphate solution and gain of phosphate by the bicarbonate solution. The total amount of phosphate lost or gained by the external media is shown in columns 5 and 8.

That the magnitude of these changes is too big to be explained on the basis of simple mixing of diffusible phosphate between fluid space in the bone and the external medium can be shown by calculating what should be the initial concentration of diffusible phosphate in the fluid space of the bone and the volume of that space, to produce changes of this order. Suppose the diffusion space in the bone has a hypothetical volume v and a concentration of diffusible phosphate C. Let the volume of the medium surrounding the bones be V, and let C_1, C_2 be the initial, and C_3, C_4 the final phosphate concentrations of the two different media in which the bones have been equilibrated. Then for a diffusion equilibrium it can be deduced from the original calculation of Eggleton (1930) that:-

Table 3

CHANGE IN THE INORGANIC PHOSPHATE CONTENT OF SALINE MEDIA DUE TO IMMERSION OF BONES

Serial No.	Wt. of bones (approximately) mg.	Phosphate saline concentration of inorganic P		Total loss of P from the surrounding medium µgP	Bicarbonate saline concentration of inorganic P		Total gain of P by the surrounding medium µgP	Hypothetical diffusion space in the bone	
		Initial µgP/ml.	Final µgP/ml.		Initial µgP/ml.	Final µgP/ml.		Phosph. concentration µgP/ml.	Volume ml.
1	2	3	4	5	6	7	8	9	10
1	200	85·6	71·6	42·0	0·5	4·8	12·9	21	0·8
2	200	85·6	60·0	76·8	0·5	8·1	22·8	20	1·9
3	250	83·2	55·5	83·1	0·7	9·6	26·7	21	2·4
4	250	83·2	71·6	34·8	0·7	6·7	18·0	29	0·8
In the following experiments the saline media were chloroform saturated.									
5	200	75·1	64·3	31·4	0·7	10·6	29·7	36	1·1
6	150	75·1	64·3	31·4	0·7	8·8	24·3	33	1·0
7	175	90·6	66·3	72·9	0·8	12·9	36·3	31	2·1
8	250	90·6	63·8	80·4	0·8	8·3	22·5	20	1·9

Duration of each experiment = 17 hours. Volume of saline = 3 ml.

$$C = \frac{C_1 (C_3-C_4) - C_3 (C_1-C_2)}{(C_3-C_4) - (C_1-C_2)} \quad \text{.....(1)}$$

$$v = \frac{V (C_1-C_3)}{C_3-C} = \frac{V (C_4-C_2)}{C-C_4} \quad \text{.....(2)}$$

C can also be determined graphically by plotting the final phosphate concentration in the two media against the initial concentrations. The point of intersection of the straight line joining the two plotted points with the straight line passing diagonally through the origin of the axes gives the value of C. This is shown for 4 experiments in Fig. 7.

Columns 9 and 10 of the table give the values of C and v obtained algebraically from equations 1 and 2.

The average specific gravity of the bone preparations employed, as already mentioned, was of the order of 1·24. Therefore, the actual volume of the bones in no experiment exceeded 0·2 ml. The figures in column 10 of the table are several times bigger than the actual volume of the bones. This strongly indicates that phosphate of the mineral phase of the bones have been involved in the exchange.

These experiments were similar to those of M.G. Eggleton (1933) with, however, the difference that no calcium salt was used in the preparation of the salines. Yet the phenomenon observed was exactly similar.

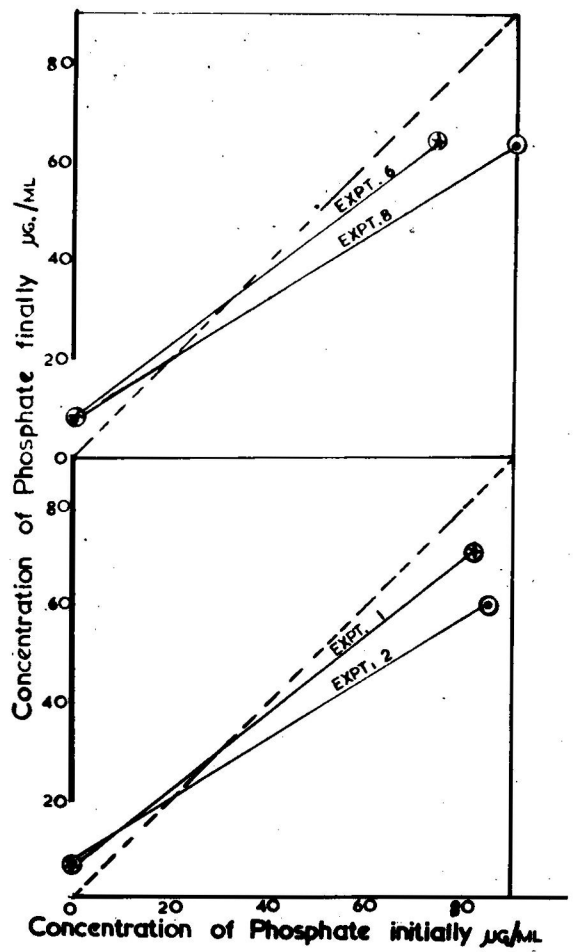

Fig. 7.

Graphical determination of inorganic P content of hypothetical bone diffusion space.

Each experiment provides two points on the graph (final concentrations of inorganic P in the two media plotted against initial concentrations). A straight line is drawn between these two points. The point of intersection with the diagonal (broken line) gives the P of the diffusion space (see text page 58).

The amount of phosphate which entered into the bones varied in different experiments from 31 to 83 µg of P. If the watery fraction of the bones amounted to 50% of the bone volume, then at the most 6 to 7 µg P could still be in a soluble form, but the remaining 25 to 75 µg P must have been deposited in an indiffusible, insoluble form. If this deposition were to be as $Ca_3(PO_4)_2$, 50 to 150 µg Ca would be required to accompany the phosphate. There was obviously no source to provide this amount of Ca within the system. It seems, therefore, justified to postulate that the phosphate ions have been fixed in the bones in exchange with some other anion, carbonates being most likely.

It should, however, be pointed out that the data in column 8 of the table representing phosphate turned out from the bones into the bicarbonate solution are perhaps explained in terms of calcium phosphate passing into solution. The largest value of phosphate that came out of the bones in the experiments was 36 µg.P. If this was due to bone $Ca_3(PO_4)_2$ passing into solution 72 µg, Ca must have passed into solutions at the same time. The ion product of calcium phosphate when 36 µg P and 72 µg Ca are present in 3 ml. of the solution, calculated in the manner indicated by Holt, La Mer and Chown (1925) at pH 7·3 is $10^{-27·04}$. The ion product calculated for the experiment in which the phosphate that came out was least, would be

$10^{-29\cdot4}$. The solubility product of calcium phosphate as estimated by Holt et al (1925) is $10^{-27\cdot2}$ at 38°C. The expected values of calcium phosphate ion product in respect of the experimental data in column 8 of the table thus do not exclude the possibility of bone calcium phosphate dissolving into solution.

The phenomenon investigated in these experiments is not inhibited by chloroform and is clearly different from the 'inorganic mechanism' of bone salt deposition described by Robinson, Macleod and Rosenheim (1930).

EXPERIMENTS OF GROUP 2

The result of the second group of experiments, in which the bones were analysed for their carbonate and phosphate contents, after having been allowed to remain in the phosphate and bicarbonate solutions for different lengths of time, is given in Table 4.

The carbon dioxide content of bones soaked in bicarbonate solution is consistently greater than the carbon dioxide content of bones soaked for the same length of time in phosphate solution. Experiments lasting for the same length of time are grouped together in the table. The mean difference in carbon dioxide content has been calculated for each such group along with its standard deviation. Application of Student's t test shows that

CARBON DIOXIDE AND INORGANIC PHOSPHATE CONTENT OF BONES SOAKED FOR VARYING PERIODS IN BICARBONATE OR PHOSPHATE SALINE.

Duration of Experiment Hours	CO_2 content of bones soaked in Bicarb.soln. cc./gm.bone	CO_2 content of bones soaked in Phosph.soln. cc./gm.bone	Difference of CO_2 content cc./gm.bone	Phosphate content of bones soaked in Phosph.soln. mg.P/gm.bone	Phosphate content of bones soaked in Bicarb.soln. mg.P/gm.bone	Difference of Phosph.content mg.P/gm.bone
22 to 24	10.64	9·12	1·52	46·28	41·43	4·85
	7·68	5·13	2·55	31·42	27·36	4·06
	9·09	6·99	2·10	36·92	32·89	4·03
	9·62	5·45	4·17	33·27	31·91	1·36
	9·43	6·54	2·89	40·51	36·39	4·12
	8·56	6·09	2·47	36·86	36·02	0·84
	10·90	8·36	2·54	46·48	43·29	3·19
	12·65	10·78	1·87	60·60	57·12	3·48
	10·39	8·32	2·07	47·86	43·66	4·20
Mean			2.46			3·35
Standard Deviation			±0·76			±1·36
't'			9·7			7·4

these differences for experiments of duration 4, 12 and 22 to 24 hours are all significantly different from zero. For experiments of zero duration the mean difference is zero.

Bones soaked in phosphate solution have shown higher phosphate content than corresponding bones soaked in bicarbonate solution in all experiments of the 12 hour and 22-24 hour duration. Departure from this finding is seen in the experiments of 4 hour duration. Here some of the differences are negative, one value is unusually high and the mean of the differences is not significantly different from zero. Difference in the carbon dioxide content of the bones in this set of experiments was significant and in the correct direction. Failure to observe significant differences in the phosphate content is ascribed to the larger margin of error of phosphate estimation already remarked upon. For zero duration, as expected, the phosphate content of the bones does not show any significant difference.

In Table 5, the mean differences in the carbon dioxide and phosphate contents of the bones are expressed in millimole units per 100 gm. of fresh bone. The data from this table is represented graphically (Fig. 8). Here the vertical lines extending above and below the points on the graph indicate the standard deviation at each point, and the smooth lines drawn free-hand show the time

Table 5.

DIFFERENCES IN THE CARBON DIOXIDE AND INORGANIC PHOSPHATE CONTENT OF BONES AFTER IMMERSION IN PHOSPHATE OR BICARBONATE SOLUTIONS.

Duration of Experiment Hours	No. of Observations	Difference in CO_2 or Phosphate content millimole/100 g. bone		't'
		Mean	Standard Deviation	
		Difference in CO_2		
0	4	0·36	± 1·26	0·57
4	8	7·4	± 2·84	7·4
12	4	10·0	± 1·49	13·5
22 to 24	9	11·08	± 3·42	9·7
		Difference in Phosphate		
0	4	1·61	± 2·9	1·1
4	8	2·58	± 6·77	1·1
12	4	10·45	± 3·77	5·5
22 to 24	9	10·8	± 4·39	7·4

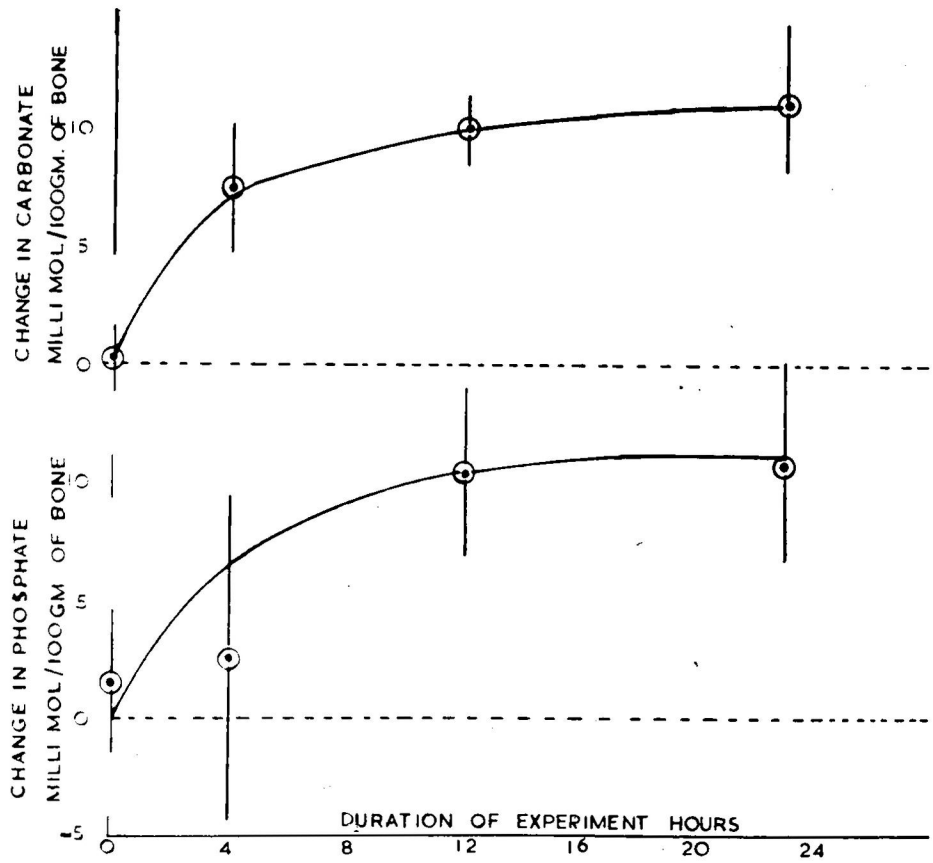

Fig. 8.

Changes in the composition of bone immersed in isotonic bicarbonate and phosphate solutions for varying periods.

The points represent the mean value of the difference in carbon dioxide or in inorganic phosphate contents between the differently treated bones. The vertical lines extending above and below the points indicate standard deviations from the mean.

course of the changes in the composition of the bones. The two lines run almost parallel, i.e. when the bones of one side have gained in carbonate the opposite bones have gained phosphate equimolarly. The molar ratio P : CO_2 of the solutions in which the bones were placed was 0·74, yet the molar ratio of the change produced in the bones was nearly 1. This would not have been so if the changes were merely due to fluid spaces in one set of bones being filled up with bicarbonate solution while those in the other set were filled up with phosphate solution. Consideration of the final magnitude of the change also prove the same point. Maximum change produced was about 11 millimole of P or CO_2 per 100 g. fresh bone. The average specific gravity of the bone preparations being 1·24, 100 g. of bone would have a volume of 80 c.c., so that even if the entire volume of the bones were replaced by bicarbonate solution in one case and phosphate solution in the other, the changes produced would be no greater than 9·4 millimole CO_2 or 7 millimole P/100 g. of bones, because the strengths of the solutions used were 11·7 millimole CO_2/100 c.c. and 8·7 millimole P/100 c.c. respectively.

The heat treated group showed exactly the same phenomenon. The observed phenomenon is, therefore, purely physicochemical.

DISCUSSION

The inorganic composition of bone is variable. From analysis of bone ash from various animal species, Morgulis (1931) expresses the average composition as:

$Ca_3(PO_4)_2$ = 77·2%
$CaCO_3$ = 11·8%
$Ca(OH)_2$ = 3·13%

One of the main reasons why bone salt is not regarded as a simple mixture of several components is the instability of $Ca_3(PO_4)_2$ in water. Basset (1917) has shown that as a hydroxy apatite $3Ca_3(PO_4)_2\ Ca(OH)_2$, it is stable over the biological range of pH. X-ray spectrography has revealed that bone salt has a crystalline structure of the apatite series of minerals. Differences of opinion exist (Huggins, 1937) as to whether the principal constituent is a Carbonate apatite, $n.Ca_3(PO_4)_2 \cdot CaCo_3$ n = 2 to 3, or a Hydroxy apatite, $3Ca_3(PO_4)_2 \cdot Ca(OH)_2$ with $CaCO_3$, or a Multiple apatite, $n.Ca_3(PO_4)_2.CaX$, where X may be CO_3, $(OH)_2$, possibly by Cl_2, SO_4, F etc.

X-ray analysis does not show any pattern of $CaCO_3$ crystals as such (Roseberry, Hastings and Morse, 1931). Carbonate is either present as a part of the apatite lattice (Carbonate apatite or Multiple apatite) or in a state of adsorption on the surface or solid solution in the substance of

Hydroxy apatite.

The investigations of the present worker indicate that carbonate should be present in a form in which exchange with phosphate is possible. It is difficult to imagine replacement of CO_3 in $CaCO_3$ by PO_4 because this would mean solution of the carbonate and reprecipitation as phosphate. But isomorphous replacement of an anion group in an apatite lattice is known to occur (Hendricks, Jefferson and Mosley, 1932). Therefore, the present work supports the view that carbonate is a part of the crystal lattice. The view is compatible with the observed exchange ratio of 1 : 1.

Since the conclusion of the work reported here, a short communication by Hodge, Neumann, Osterhoudt, Mulryan and Bale (1953) has appeared in the communications of the International Physiological Congress Montreal 1953, saying that much of the carbon dioxide of bone is fixed by a heteroionic exchange of CO_3 for the surface phosphate groups in the mineral crystals. The details of their experiments have not appeared. The study was on powdered bone ash suspended in solutions of bicarbonate containing C^{14}.

It is generally assumed that carbon dioxide in bone is present in the form of carbonate. Neuman, Neuman, Main, O'Leary and Smith (1950) have produced evidence of direct competition between OH, HCO_3 and F ions for combining sites in the bone and are of

the opinion that CO_2 is bound to the mineral by a single bone B-O-CO_2.H. where H may be replaced by Na or other cation.

Carbonate is the most variable component of the inorganic bone substance. Carbonate content of bone has been known to increase with age (Shear and Kramer, 1928; Neal, Palmer, Eckles and Guillickson, 1931). Vogt and Tømsager (1948), having analysed samples of human bone of different age groups, have proposed that carbonate content changes inversely with phosphate: CO_3 being substituted with PO_4 up to age 40-50, followed by a reverse substitution.

Howland, Marriott and Kramer (1926), and Shear and Kramer (1928) found that rachitic bones of rats or man contained more carbonate than normal bone. But Sobel, Rockenmacher and Kramer (1945) have now found that when rickets is produced by high phosphorus and low calcium diet, the CO_3:Ca ratio in the bones is actually lowered. Only when rickets is due to high Ca and low P diet is there an increase in CO_3:Ca ratio in the bones. They propose that there is a reciprocal relationship between serum inorganic phosphorus and bone carbonate.

Several workers have shown that in acidosis bone carbonate is reduced. Goto (1918) fed rabbits with 25 to 50 ml. $\frac{N}{4}$ HCl per day and found that the carbon dioxide content of their bones was

diminished.

Irving and Chute (1932) studied the effect of acid feeding on bone carbonate of rats and guinea-pigs and found on average 11% depletion of carbon dioxide.

In these instances one is casually apt to think in terms of an acid acting on the bone and releasing carbon dioxide. In all these experiments the bones have been acted upon by a medium of higher chloride content rather than one of a lower pH. Replacement of carbonate by chloride would explain the phenomenon much better than decomposition of bone mineral. Irving and Chute (1930) do record that the decomposition of bone minerals which occurs during the administration of acid does not follow the proportions of the chief substances present, but the carbon dioxide component is really more labile. It is hard to explain how this extra carbon dioxide could leave the bone without an equivalent amount of cation leaving with it unless it had been displaced by another anion.

According to the theory of Multiple apatite structure of bone mineral, as represented by $n.Ca_3(PO_4)_2.CaX$, the X position may be occupied by many anions. If the X position is imagined to be entirely occupied by a carbonate group, the result is carbonate apatite. Analysis of bone almost always shows an excess of basic over acid equivalents than that required by a carbonate

apatite formula, $n.Ca_3(PO_4)_2.CaCo_3$, which shows that either bone salt is partly present as a Hydroxy apatite or other anions are present. Upon fossilization of bone the carbonate group is known to be replaced by fluorine (Hendricks, Hill, Jacob and Jefferson, 1931). So it is reasonable to consider chloride replacing bone carbonate in the acidotic conditions considered. Dickens (1941) has found that the bone substance contains a hitherto unsuspected store of citric acid, which may constitute some 70% of that contained in the whole body. He has observed that the citrate content of bones is extremely variable and suggests that it is present in a readily available form. Thus into the picture of labile anions in the bones which the present writer is trying to draw, one physiologically important organic acid comes in. There has also been a suggestion by Irving and Chute (1932) that during strenuous muscular exercise bone carbon dioxide may be discharged from accumulation of lactic acid. The process of ammonia formation in the kidneys as an acid neutralizing mechanism has, according to Fiske, Goodell, Hathaway and West (1926), a time lag. On this ground Irving and Chute believe that lability of bone carbon dioxide may be of physiological significance in sudden change of acid base equilibrium.

It has been mentioned that the maximum difference induced in phosphate or carbonate content of

the two differently treated sets of bone in the experiments of the present worker was 11 millimole/100 g. of fresh bones. Therefore, according to these experiments, the replaceable anions in the bones amounted to 5·5 millimoles/100 g. fresh bone. It is interesting to point out that the maximum measure of lability of bone carbon dioxide arrived at by Irving and Chute (1932) as a result of their observations was exactly similar. Their figure was 0·0067 Mole/120 g. of bone, i.e. 5·6 millimole/100 g. of bone.

There is evidence also that the bone carbon dioxide is labile enough to respond fairly rapidly to alterations in alveolar carbon dioxide tension. Ferguson, Irving and Plewes (1929) observed that the carbon dioxide blown off from a cat by over-ventilation exceeds the amount produced from metabolism during the period. Of the extra carbon dioxide blown off not more than 10 to 30% can be accounted for by decrease in the carbon dioxide content of soft tissues. They suspected the bones to be the source of this unexplained extra carbon dioxide. Conversely, Irving, Ferguson and Plewes (1930) found that if the respiratory elimination of carbon dioxide is retarded by the presence of 10 to 12% carbon dioxide in the inspired air, the carbon dioxide retained in the body remained largely unaccounted if the capacity of only the soft tissue to hold carbon dioxide is considered.

They analysed the bones but did not consider their result fully convincing though in most cases examined the bone carbon dioxide did show change in the expected direction.

Very recently Freeman and Fenn (1953) investigated the location and extent of the carbon dioxide stores in the body of rats living in an atmosphere low in oxygen or high in carbon dioxide for a number of days, and have produced fairly conclusive evidence that the bones of the body participate in the equilibration process to altered carbon dioxide tension in the lungs.

Calculated on the basis of their data, 1 mm. mercury rise in the alveolar carbon dioxide pressure increases the total carbon dioxide store in the body of a 70 kilo man by 800 c.c. The quantity is too large to be accommodated in the soft tissues of the body.

The carbon dioxide capacity of human blood is only 0·5 c.c. per mm. of mercury change in the tension of carbon dioxide. The extracellular fluid may be considered to have about the same carbon dioxide capacity as blood. But the carbon dioxide capacity of intracellular fluid is about one-third that of blood (Wallace and Hastings, 1942). The proportions of the extra and intracellular fluids in the body of a man have been indicated in Fig. 1. A simple calculation will show that the soft tissues of the body cannot account for more than 150 c.c. of

the 800 c.c. CO_2 mentioned above. The bone mineral is then the only site left which can account for the remaining 650 c.c. of carbon dioxide. This is the main implication of the work of Freeman and Fenn (1953).

In these experiments of Freeman and Fenn (1953) the bones of one group of rats lost 11% of their normal carbon dioxide and of the other group gained 7%. The carbon dioxide content of the bones in their control groups was about 2·5 g. CO_2/100 g. bone. The magnitude of the change induced in the carbon dioxide content of the bones was, therefore, roughly 0·25 g. CO_2/100 g. bone, i.e. equivalent to 5·7 millimole CO_2/100 g. bone. This figure agrees closely with the maximum degree of the lability of bone carbon dioxide observed by Irving and Chute (1932) and equally well with the finding of the present worker that the replaceable anions in the bones amount to 5·5 millimole/100 g. fresh bone.

The graphs in Fig. 8 give a rough idea of the time taken for the changes in the carbon dioxide and the phosphate content of the bones to reach their apparent maximum. It appears that under conditions of the present experiment it took about 24 hours for the process of replacement of the carbonate or phosphate to reach a limit. The factors which decide the time course of the process seem to be obscure. In this connection it is relevant to refer to certain investigations of

Johansson, Falkenheim and Hodge (1945) and of Falkenheim, Neuman and Hodge (1947). These investigators studied the rate of interaction between bone mineral and phosphate buffers containing radioactive P^{32}. They were of the opinion that the rate of incorporation of radioactive phosphate by the bone mineral involved a diffusion process and was limited by the rate at which P^{32} could diffuse into the less accessible parts of the bone particles. This opinion was based on their finding that the amounts of P^{32} adsorbed was linearly proportional to the square root of the adsorption times. But Neuman and Mulryan (1951) now think that the slowness of the incorporation of labelled phosphate is primarily the result of a recrystallization process rather than a simple diffusion of the phosphate ion through the crystal lattice. They, however, still think that other ions, fluoride and hydroxyl ions for example, may enter the mineral crystals by a diffusion process.

The investigations on intact animals which have produced measurable alterations in the carbon dioxide content of the bones have been mostly of a more or less chronic type. In the experiments of Irving and Chute (1932) acid feeding for 3 days produced maximum possible reduction in bone carbon dioxide of guineapigs. The duration of the experiments of Freeman and Fenn (1953) varied from 10 to 31 days in one group, and from 6 to 28 days

in the other group of rats. They record that an exposure to 7% oxygen mixture lasting for only 6 hours did not produce any change in the bone carbon dioxide of rats. On the other hand, the experiments of Ferguson et al (1929) and Irving et al (1930) were of an acute nature and lasted for about 3 hours only; and yet the amount of carbon dioxide involved in the respiratory exchange was so great that the bones appeared to have participated in the exchange. The experiments of Shaw and Messer (1930) lasted for about 2 hours but the amount of carbon dioxide involved in these experiments was much smaller and could very nearly be accounted for by the changes in the carbon dioxide stores of the soft tissues only (see page 43 of the present work). Thus the time course of the process or processes by which bone carbon dioxide participates in the carbon dioxide exchanges of the body is uncertain.

The present investigation only indicates that in the substance of the fresh bones (of frogs) there exist certain anion positions which can be occupied by bicarbonate or phosphate ions if the bones are immersed in isotonic bicarbonate or phosphate salines. These replaceable anion positions probably amount to 5·5 millimoles/100 g. fresh bone; and the replacement can be complete in about 24 hours. It should be emphasized that the phenomenon observed is purely physicochemical and is not inhibited by the presence of chloroform in the

media or by previously heating the bones to the boiling temperature of the saline.

SUMMARY AND CONCLUSIONS

In vitro, experiments with preparations of fresh bones (femur and tibio-fibula of frogs) are described, the results of which indicate that in the bones there exist anion positions for which phosphate and bicarbonate ions can compete.

In one group of experiments the bones of one side were placed in 3 ml. of a saline prepared by mixing 3 ml. of an isotonic phosphate solution (270 mg.P/100 ml.) with 97 ml. of isotonic sodium chloride solution. The bones of the opposite side were placed in 3 ml. of a saline prepared by mixing 3 ml. of an isotonic bicarbonate solution with 97 ml. of isotonic sodium chloride solution. Estimation of inorganic phosphate in the media around the bones after 17 hours showed that the phosphate saline had lost and the bicarbonate saline had gained inorganic phosphate.

Quantitative assessment of the magnitude of the change indicates that the phosphate of the mineral phase of the bones had participated in the exchange. This phenomenon of phosphate exchange was not inhibited by chloroform.

No calcium salt was used in preparing the salines. The loss of phosphate from the phosphate saline was, therefore, not due to a deposition of calcium phosphate in the bones. This suggests that the phosphate ions were fixed in the bones in

exchange of some other anion, carbonate being most likely.

In the second group of experiments, the bones of one side were placed in isotonic phosphate solution and the bones of the other side in isotonic bicarbonate solution for 4, 12 and 24 hours. The bones were then analysed to determine their carbon dioxide and phosphate content.

The mean value of the difference in the carbon dioxide content of the bones soaked in bicarbonate solution and bones soaked in phosphate solution was found to reach a value of 11 millimole/100 g. fresh bone in experiments lasting about 24 hours.

The mean value of the difference in the inorganic phosphate content of phosphate and bicarbonate soaked bones was also found to reach a value of 11 millimole/100 g. fresh bone, in experiments of 24 hours duration.

It is discussed how far various reports in the literature on the lability of bone carbonate also imply an anion exchange, and it is concluded that this is a probable mechanism by which bone carbonate may participate in exchanges with body fluids and probably assist in the regulation of their carbon dioxide concentration.

PART III

RESPIRATORY ADAPTATION TO CARBON DIOXIDE ADMINISTERED IN THE INSPIRED AIR AT A FIXED RATE.

INTRODUCTION

During the steady state of a subject, carbon dioxide enters the lungs from the blood at the rate of its production in the body and is removed in the expired air at the same rate. The purpose of this part of the work was to study how the respiratory mechanism would react to preserve the steady state if extra carbon dioxide was introduced into the lungs with the inspired air.

For this purpose carbon dioxide has been added to the inspired air of the subject at an approximate rate of 120 c.c./min. to correspond roughly to an increase of 50% in the resting metabolism. The ventilation rate of the subject and the rate of elimination of carbon dioxide in his expired air have been measured before, during and after the administration of the carbon dioxide in the attempt to find out:

a) How long it takes for the ventilation rate to reach a new level under the stimulus of the carbon dioxide and on the discontinuance of this stimulus to regain the original level.

b) When does the rate of elimination of carbon dioxide in the expired air balance the rate of its production in the body and the rate of its administration to the inspired air.

c) How much carbon dioxide is dammed back within the body during adjustment to its administration

in the inspired air and given out later when the subject breathes normal air.

Beginning with the experiments of Haldane and Priestley (1905), numerous studies have been made on the respiratory effects of carbon dioxide inhalation, but only a few of them have a bearing upon the questions outlined above.

In 1905, Haldane and Priestley took it as an assumption that a subject inhaling air enriched with carbon dioxide remained in the steady state with respect to carbon dioxide.

A few years later, Campbell, Douglas, Haldane and Hobson (1913) tried to verify the assumption in two side experiments. In the first experiment the subject sat inside a specially constructed chamber and breathed into a dry gas meter. His average ventilation rate while breathing pure air was 8·6 litres per minute. The concentration of carbon dioxide inside the chamber was suddenly raised to 5%.

Litres of air breathed in successive minutes for 20 minutes were as follows: 16·4, 27·5, 35·5, 43·4, 42·3, 38·8, 36·0, 36·2, 37·4, 36·5, 37·9, 36·8, 35·9, 38·8, 39·6, 38·5, 40·2, 40·2, 40·2, 41·9.

The door of the chamber was opened and a fan started so that the chamber was cleared of carbon dioxide within 15 seconds. Litres of air breathed in successive minutes were: 19·3, 10·8, 8·5, 7·6, 9·3, 8·5, 9·3, 9·6, 9·6, 9·9, 9·6, 9·3, 9·6.

The gradual increase in the ventilation rate

from after the 7th minute till the end of carbon dioxide inhalation and the failure to regain the resting level up to 14 minutes afterwards have been considered by these investigators as suggestive of a prolonged damming back of carbon dioxide during its inhalation and a slow discharge of the dammed back carbon dioxide on breathing pure air. The data of this experiment of Campbell et al (1913) have been described above in some detail because they will substantiate a point of discussion at a later stage.

Their second experiment was of a different nature. In this experiment measurements were made of the respiratory exchange of the subject before and during the 3rd, 11th and 18th minutes of breathing air having 6% carbon dioxide in it. The respiratory quotient of the subject in each of these three intervals was found to be lower than normal. It can be calculated from their data that the rate of elimination of carbon dioxide from the body of the subject was falling short of its rate of production by 150, 130 and 60 c.c. during the 3rd, 11th and 18th minutes of the experiment respectively.

In 1914, Campbell, Douglas and Hobson prolonged the period of observation to 1¼ hours in the hope that the subject would reach a steady state of carbon dioxide equilibrium. In one series of three experiments in which the subject was inhaling a 4·5% mixture of carbon dioxide in air, the

respiratory quotient returned to normal. But in a second series of four experiments the respiratory quotient remained definitely low throughout the period of hypernoea of the subject who was inhaling 3·5% carbon dioxide in air for 1¼ hours.

Adolph, Nance and Shilling (1928) administered 5% carbon dioxide in air for ½ hour to their subjects and came to the conclusion that a steady state of carbon dioxide equilibrium could not be attained in ½ hour. The alveolar carbon dioxide pressure continued to vary in most experiments. In one or two instances alveolar carbon dioxide tension appeared to be constant while the total ventilation was still changing. The respiratory quotient returned approximately to normal only towards the later part of breathing carbon dioxide.

In these experiments all the expired air was collected from half an hour before the inhalation of carbon dioxide to one hour afterwards, in 12 samples. From analysis of these samples they were able to make rough estimates of the amount of carbon dioxide dammed back within the body and later given out. The figures varied from 400 to 2100 c.c. with one exceptionally high value of 4900 c.c.

Padget (1928) has observed that when air containing an increased amount of carbon dioxide is breathed, the maximum increase in respiration occurs only after the mixture has been breathed for

some time. But his observations showed that the length of the 'lag' varied from 2 to 7 minutes depending on the concentration of carbon dioxide in the air breathed, being longer with the higher percentages. The percentage of carbon dioxide in his experiments varied from 2 to 6%.

Dripps and Comroe (1947) have made observations with 7·6 and 10·4% carbon dioxide. They have found that the respiratory minute volume reached a plateau after 2·5 to 8 minutes if the air contained 7·6% carbon dioxide and after 2·5 to 6 minutes if the air contained 10·4% carbon dioxide.

Lambertsen, Kough, Cooper, Emmel, Loeschcke and Schmidt (1952) have considered that 8 to 10 minutes are sufficient for the ventilatory response to reach a maximum.

Duncan Weatherley (1952) records similar finding. The ventilation rate was found to reach an apparent plateau in a few minutes.

But in the above observations of Padget (1928), Dripps and Comroe (1947), Lambertsen et al (1952) and Duncan Weatherley (1952) only the ventilation rate was measured, so it cannot be said whether the subjects had reached a steady state of carbon dioxide equilibrium within such short intervals of time. This point will be further taken up in the discussion.

In the different experiments referred to in the foregoing account, the subjects have inhaled air

containing constant or almost constant percentages of carbon dioxide. Usually the percentage of carbon dioxide has been high.

This practice of fixing the percentage of carbon dioxide in the inspired air has a disadvantage. As the ventilation rate increases, the amount of extra carbon dioxide entering the lungs also increases. When pulmonary ventilation rate increases in response to an increase in the endogenous carbon dioxide, the condition is obviously different. In this latter case, the increase in the volume of the inspired air does not introduce any additional carbon dioxide into the lungs; on the contrary, it helps to dilute the extra endogenous carbon dioxide which enters the lungs from the blood.

It is on this line of thought that a new method of administering the carbon dioxide has been devised in the present investigation. The rate of addition of carbon dioxide to the inspired air has been fixed and not its percentage. Carbon dioxide has been administered in the present experiments by adding it to the inspired air at the rate of 120 c.c. per minute irrespective of the volume of air breathed, the object being to create a condition similar to that produced by an approximately 50% increase in endogenous carbon dioxide.

METHODS

The experimental procedure involved methods for the following:

1. Recording of pulmonary ventilation rate continuously and in a quantitative manner.
2. Delivery of carbon dioxide at the rate of 120 c.c./minute to the inspired air.
3. Collection of representative samples of the expired air and analysis of samples.

RECORDING OF PULMONARY VENTILATION

Haldane and Priestley (1905) employed a body plethysmograph to obtain a quantitative record of pulmonary ventilation. Recently Cross (1949) adopted the same method for studying respiratory activities of new born babies. But it was thought that a body plethysmograph for an adult would be inconveniently large, and on account of large size and risks of leakage would not be accurate enough for the present purpose.

Breathing simply into a gas meter and taking readings at short intervals was found unsatisfactory. The meter offered a variable degree of resistance, and the movement of its pointer, with interruptions at each inspiration, was difficult to follow accurately. These difficulties were overcome by the method described below.

Fig. 9 shows the diagram of the apparatus employed. The subject breathed through a

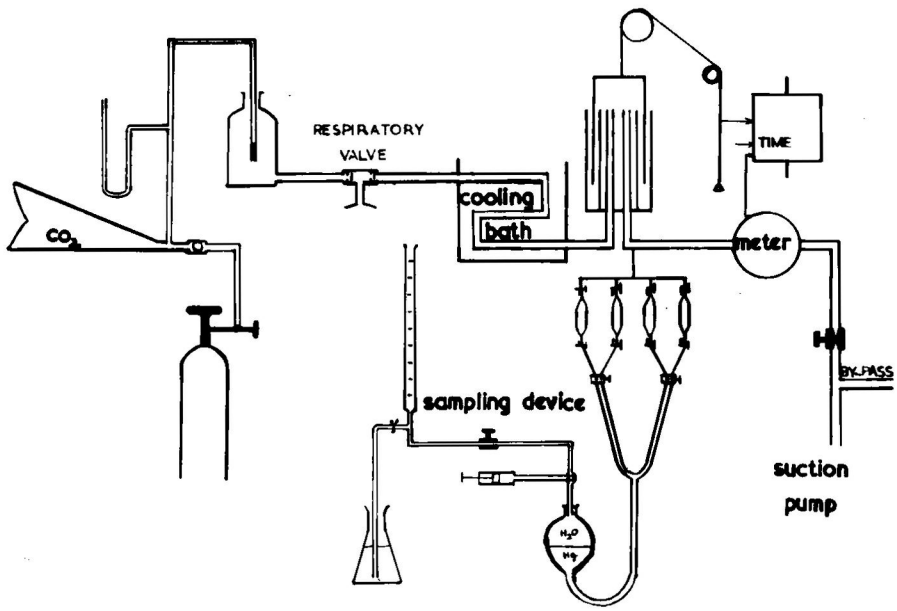

Fig. 9.

Diagram of the apparatus for a continuous measurement of the pulmonary ventilation rate. The diagram also shows the arrangements for the administration of carbon dioxide and for the collection of samples of expired air.

respiratory valve into a meter. But between him and the meter was a spirometer bell. To maintain a continuous flow through the meter and to overcome its resistance, a suction pump was used. During expiration, air entered the spirometer bell faster than it was withdrawn by the pump and the bell ascended. During inspiration, the bell fed the pump. The height of the bell was continuously recorded on the smoked paper of a moving drum along with a time tracing and a signal from the meter, arising from an electric contact made by its pointer once during each rotation. The meter used was a wet meter of Gorman Siebe make, and one complete rotation was 2·5 litres.

The rate of suction of air through the meter was regulated so that it was nearly equal to the ventilation rate of the subject. For this purpose the suction pump was provided with a by-pass. With a throttle between the by-pass and the meter, the rate of suction through the latter was so adjusted that the tracing of the spirometer bell was not allowed to pass beyond the width of the smoked paper. This procedure also guarded against the spirometer bell being completely emptied of air. If this was allowed to happen the negative pressure of suction would act directly on the respiratory valve and air from the outside atmosphere would be drawn in.

In order to ensure a fairly constant temperature a cooling bath was provided in the path of the

expired air before the bell. Inside the spirometer the inlet and outlet tubes were specially elongated, and the space around filled up with water to reduce dead-space.

The kind of record obtained from an experiment is shown in Fig. 10. The procedure of calculating the pulmonary ventilation rate from the record was as follows: the record was placed under a glass plate with millimetre square rulings marked on it. The distance between the successive signals from the meter was read. The time tracing gave the time scale and so the intervals of time during which 2·5 litre volumes of air had passed through the meter were known. The actual volume of air expired during each of these intervals was 2·5 litres plus or minus a correction for the change in the position of the spirometer bell. Corresponding to each signal mark from the meter, the position of the spirometer bell was read from the record. A change in the level of the bell of 14 mm. was equal to a volume change of 300 c.c. A change in the downward direction of the tracing corresponded to a rise in the level of the bell. The amount of air collecting inside the bell added to 2·5 litres was the volume of expired air during the interval. If the bell had partially emptied during an interval, the loss of volume inside it was subtracted from 2·5 litres to get the volume of the expired air. The corrected volumes of expired air, obtained as

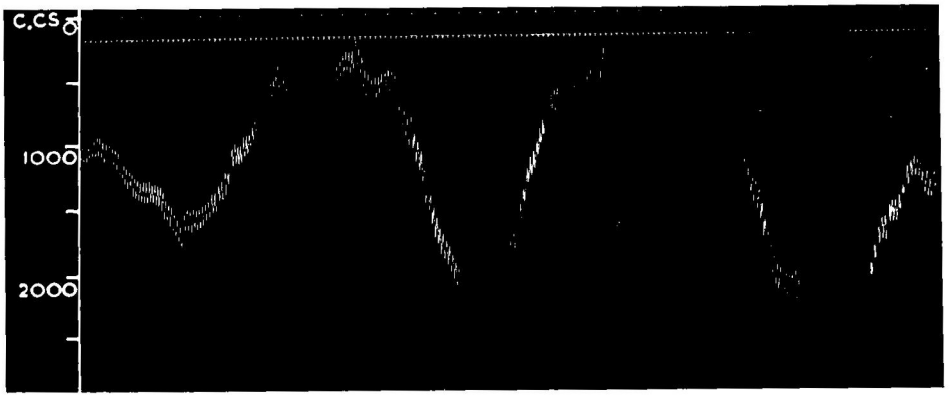

Fig. 10.

Specimen of kymograph record of spirometer bell movement obtained using the apparatus shown in Fig. 9.

Tracings in order from the top: 2·5 litre signals from the gas meter; time, 5 seconds; position of the spirometer bell.

Subject I.C. 1.3.54.

explained above, divided by the duration of the intervals, gave the average rates of ventilation.

To calculate tidal volume, the volume of air expired at each interval was divided by the number of expiratory movements during the interval.

The calculated rates of pulmonary ventilation were minute volumes of expired air at prevailing atmospheric pressure and saturated with water vapour at the temperature of the spirometer bell.

This temperature in the present experiments was about 20°C.

Since 2·5 litres is roughly five times the volume of a normal breath, the ventilation rates measured by the above method are averages over 5 to 6 breaths.

It was considered that a measurement of the ventilation rates over fewer number of breaths would not serve any useful purpose. Pulmonary ventilation is a discontinuous process and liable to fluctuate from breath to breath. To look for any significant change of pulmonary ventilation one has, therefore, to rely on a mean value. A mean value over approximately 5 breaths appeared to be suitable for the present investigation.

Record of a Respiration Pump

The reliability of the above method of recording pulmonary ventilation has been tested by placing a respiration pump in the place of a subject. Fig. 11 shows the kind of record produced on the

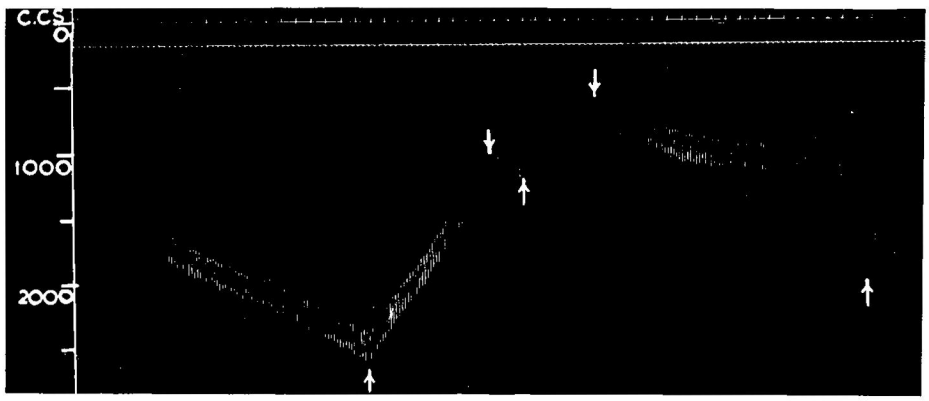

Fig. 11.

Specimen of kymograph record of spirometer bell movement obtained using the apparatus shown in Fig. 9, the subject being replaced by a respiration pump. (500 c.c.-stroke volume).

Tracings in order from the top: 2·5 litre signals from the gas meter; time, 5 seconds; position of the spirometer bell.

Arrows indicate adjustments of the rate of suction of air from the spirometer bell.

smoked paper. The tracing of the position of the spirometer bell is characteristically regular.

During the experiment a few abrupt changes were produced in the position of the spirometer bell by deliberately altering the rate of suction of the air from it. The method of applying corrections for changes in the level of the spirometer in the calculation of the ventilation rates takes account of these manipulations. This is shown by the next figure (Fig. 12). In this figure the ventilation rate of the respiration pump is shown graphically. The graph shows a certain amount of irregularity. A measure of these irregularities is obtained by calculating the mean value of the ventilation rate and its standard deviation. The mean value of the ventilation rate over the period of observation was 11·08 litres/min. with a standard deviation of ± 0·24 litres/min. (coefficient of variation = 2·2%). Therefore, assuming that the respiration pump produced a perfectly steady ventilation, the method employed to measure the ventilation rate could be relied upon to give results with an average variability not exceeding 2·5%.

METHOD OF DELIVERING CARBON DIOXIDE

The diagram in Fig. 9 shows the method adopted for administering carbon dioxide during the experiments. Carbon dioxide was stored in a Douglas bag at a pressure of about 10·5 cm. of Brodie's fluid.

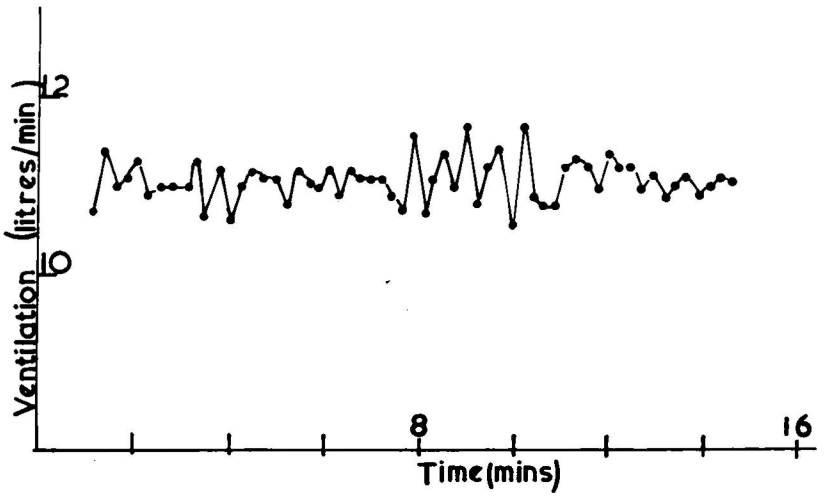

Fig. 12.

The ventilation rate of the respiration pump calculated from the record shown in Fig. 11.

When required, it was allowed to run into a hopper bottle through a piece of capillary glass tubing which acted as a throttle to produce the desired rate of flow. The hopper bottle was large enough to hold air sufficient for one breath. One of its openings was connected to the inspiratory end of the respiratory valve and the other left open to the atmosphere.

In a preliminary experiment the rate of delivery of carbon dioxide from the bag was measured by observing the displacement of water inside a burette inverted in a cylinder of water. By this method a suitable length of capillary tubing was selected to give a delivery rate of approximately 120 c.c./min.

METHOD OF SAMPLING EXPIRED AIR AND ANALYSIS OF SAMPLES

Sampling device

Fig. 9 also shows the device used for sampling expired air. The samples were taken as the air left the spirometer bell. The device consisted of 4 tonometers, in parallel, connected to a common reservoir. To collect a sample the mercury from a tonometer was allowed to drain into the reservoir. Above the mercury level the reservoir contained water. As mercury collected at the bottom, water was displaced into a burette. The escape of water from the reservoir into the burette was watched and controlled with the throttle, shown in the diagram, so as to make the rate of collection

of sample nearly proportional to the flow of air through the meter; a fixed number of c.c.s of expired air being collected for each rotation of the meter hand. The connection between the burette and the reservoir was through a 3-way tap, which permitted a small syringe to be brought into use to clear out traces of dead space in the connecting tubes above the tonometers in the final stages of charging them with mercury.

Analysis of samples

The samples were analysed for the concentration of carbon dioxide in them by the manometric method of Van Slyke, Sendroy and Liu (1932). The samples were large enough for duplicate determinations to be made.

The sample to be analysed was introduced into the Van Slyke's apparatus. Ps readings were taken at 50 c.c. volume. Carbon dioxide was absorbed by an alkali and residual gases were expelled from the chamber. Next an acid was introduced to liberate the carbon dioxide which had been absorbed by the alkali. The carbon dioxide was finally re-absorbed by a second introduction of alkali to determine its partial pressure, P_{CO_2}, by difference. P_{CO_2} readings were taken at 2 c.c. volume.

The concentration of carbon dioxide found in the sample multiplied by the average rate of ventilation during the period of sampling gave the rate of elimination of carbon dioxide for the

period.

When the concentration of carbon dioxide in the air passing out of the spirometer was steady, it was immaterial whether the rate of sampling was or was not proportional to the ventilation rate.

But the main interest of the present study was in the periods during which the rate of carbon dioxide elimination changed. Here the accuracy of the calculated values of the rate of carbon dioxide elimination depended on how exactly the rate of sampling was proportional to the ventilation rate. An attempt was made to make the sampling nearly proportional to the flow of air through the meter in the manner already described. But it was not considered very satisfactory.

A check on the delivery rate of Carbon Dioxide

As already described, the method of delivering carbon dioxide was to allow the gas to enter a hopper bottle through a capillary tube at the rate of approximately 120 c.c./min. Reliance was placed on the fact that, due to its higher density than air, the gas would collect at the bottom of the bottle and be drawn into the lungs with the inspired air. It required to be seen whether this would be so in practice. Accordingly a number of test experiments were performed employing the respiration pump in place of a subject. Carbon dioxide was allowed to enter the hopper bottle and samples of air coming out of the spirometer were

Table 6

RATE OF FLOW OF CO_2 THROUGH THE RESPIRATION PUMP AND THE SPIROMETER BELL

Pressure of CO_2 supply cm. of Brodie's fluid	Temperature °C.	Ventilation rate litres/min.	Rate of flow of CO_2 c.c./min.
11·4	19	9·2	114
11·3	22	9·7	112
10·0	20	9·3	116
			118
		9·7	118
			120
10·7	17	7·0	118
			114
		4·9	114
			112
		10·8	124
10·7	17	5·6	124
			119
		5·7	123
			127
			Mean = 119

The figures for CO_2 include atmospheric CO_2.

collected and analysed for carbon dioxide.

Table 6 shows the result of these experiments. The pump was worked at several different rates to cover the range of ventilation rates expected in the subjects. The samples were collected after at least 20 litres of air had passed through the apparatus.

The result indicates what could be expected of the human subjects on regaining a steady state during administration of carbon dioxide. The mean value of the rate of flow of carbon dioxide through the apparatus was 119 c.c./min., including carbon dioxide normally present in air. So on an average a subject would be expected to eliminate 115 c.c. CO_2/ min. in excess of his metabolic output if he had reached a steady state while being administered carbon dioxide in the manner described.

SUBJECTS OF EXPERIMENT

Most of the observations were made on two subjects. Subject I.C.; age 17 years, height 73 inches, weight 10 stones, and subject S.M.G.; age 38 years, height 65 inches, weight 8 stones. A few observations were made on subject P.E.; age 51 years, height 64 inches, weight 10 stones. (The third subject was not in his normal health. Shortly afterwards he manifested signs of cardiac trouble.)

No attempt was made to obtain a basal condition in the subject, but observations were made under

resting conditions and 2 hours after any preceding meal.

The subject sat comfortably in an easy chair and relaxed for 30 minutes before putting on the mouthpiece of the respiratory valve. He was allowed 5 minutes to get used to the mouthpiece and another 5 minutes with noseclips on. Thereafter the expiratory end of the respiratory valve was connected to the spirometer bell through the cooling bath, the suction pump was switched on and his ventilation rate recorded.

RESULTS

The Ventilatory Response

The method of computing the ventilation rate from the kymographic record obtained during an experiment has been explained in the previous chapter. The analysis of the record furnishes a series of values of the average ventilation rate over each successive 2·5 litre intervals. These values are plotted on a graph paper against time. The graph then represents quantitatively the behaviour of the pulmonary ventilation rate of the subject during the period of observation.

Three such graphs are shown in Figs. 13, 14, and 15. They show the normal variations in the resting pulmonary ventilation rates of the three subjects of the present experiments. The ventilation rate was not steady in any of the subjects, and the degree of irregularity varied greatly from one subject to the other. A comparison with Fig. 12, the graph representing the ventilation produced by the respiration pump, would show that these irregularities were real variations of the pulmonary ventilation and not artefacts due to faulty recordings. The irregularities are least in Fig. 15. In this record the ventilation rate has a mean value of 5·04 litres/min. and a standard deviation of ± 0·40 litre/min. The coefficient of variation, therefore, amounts to 8%; this is more than three times the coefficient of variation found with the

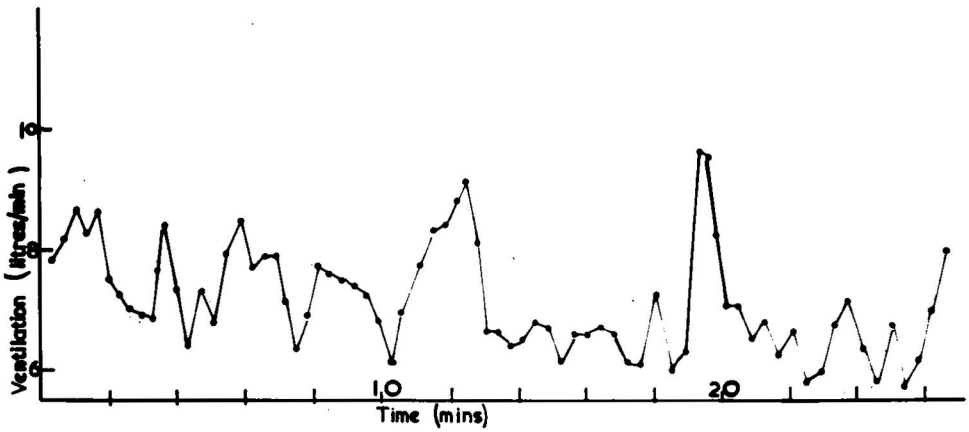

Fig. 13.

The usual character of the pulmonary ventilation in Subject I.C. (1.3.54)

The kymographic record of the measurement is shown in Fig. 10.

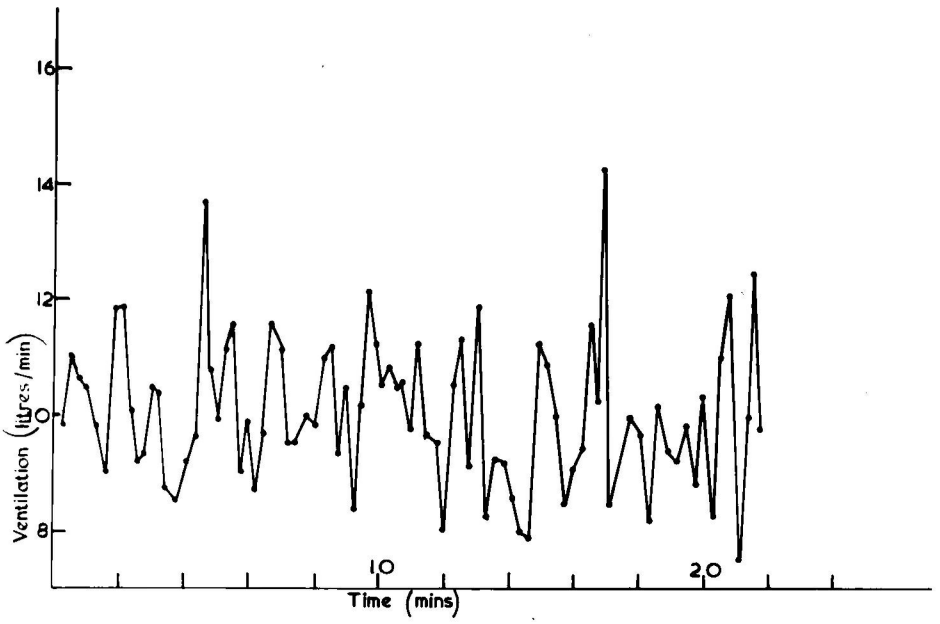

Fig. 14.

The usual character of the pulmonary ventilation in Subject P.E. (25.2.54)

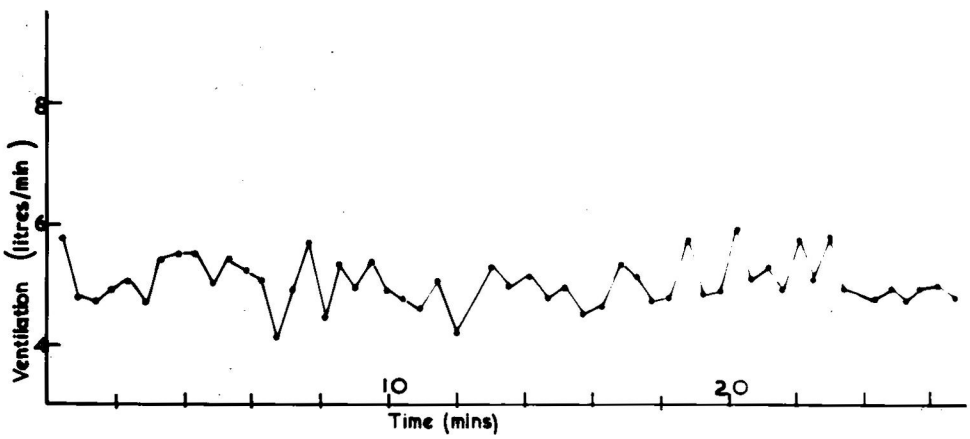

Fig. 15.

The usual character of the pulmonary ventilation in Subject S.M.G. (26.2.54)

ventilation record of the respiration pump. The variability of the rate of pulmonary ventilation seen in Figs. 13 and 14 are very much greater and statistical comparison with Fig. 12 would be superfluous. It should be mentioned here that such extreme irregularities as are seen in Fig. 14 are probably not physiological. The subject of this experiment was later on found to be suffering from a mild degree of heart failure.

The graphs in Figs. 13, 14 and 15 illustrate another fact. They show that, though the ventilation rate at one moment may be widely different from the rate at another moment, the mean level of the graph over the period of observation tend to be even.

The next three graphs in Fig. 16, 17 and 18 are representative of the type of effect produced on the pulmonary ventilation by the administration and withdrawal of carbon dioxide. The ventilatory pattern characteristic of each subject is unchanged. The same irregularities are there, but the effect of carbon dioxide is shown by a general shift of the level of the curves.

The irregular nature of the ventilatory pattern makes it difficult to discern clearly the effect of carbon dioxide in single records. A method has to be adopted by which the average effect of a number of observations on the same subject could be brought out. Superimposition of the graphs obtained from

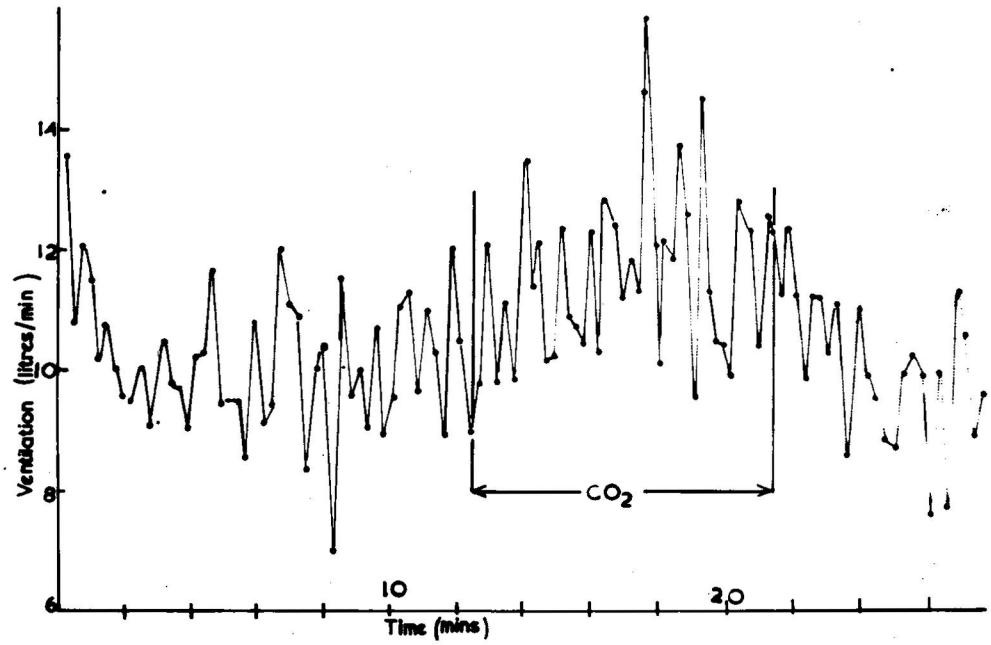

Fig. 16.

The effect of carbon dioxide on pulmonary ventilation. Subject P.E. (5.3.54)

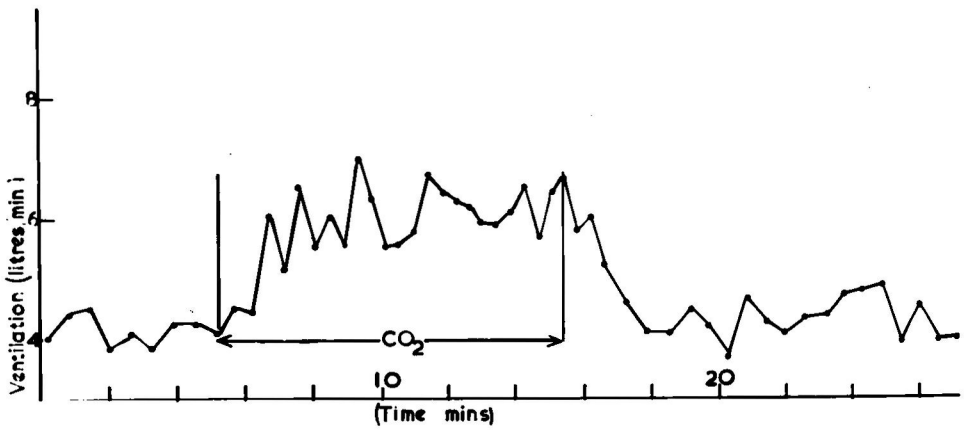

Fig. 17.

The effect of carbon dioxide on pulmonary ventilation. Subject S.M.G. (16.3.54)

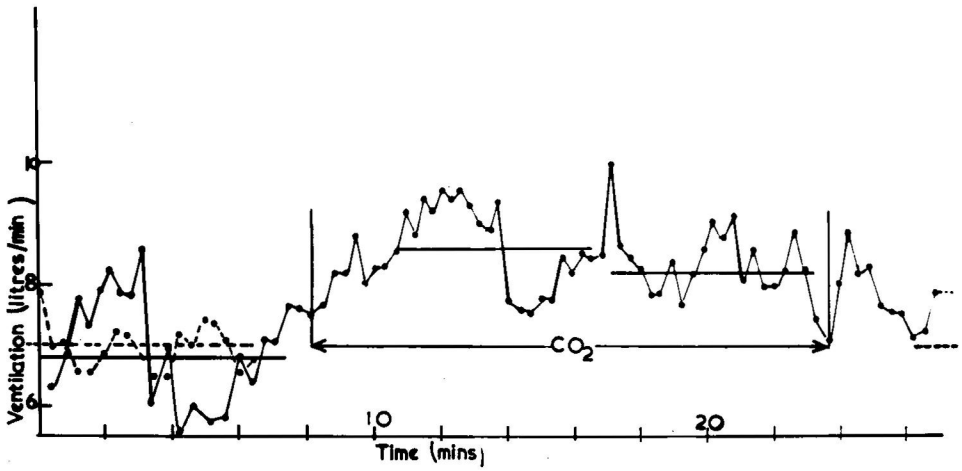

Fig. 18.

The effect of carbon dioxide on pulmonary ventilation. Subject I.C. (13.5.54)

The graph in broken line is the continuation of the ventilation curve beyond the right hand edge of the figure.

The horizontal lines (full and broken) indicate periods during which samples of expired air were collected for carbon dioxide estimation.

a number of separate experiments on the same subject has proved suitable for the purpose.

The superimposition of the graphs has been done in the following manner: the average ventilation rate during the two minutes immediately preceding the administration of carbon dioxide is taken as a base line for the graph. To superimpose a number of graphs their base lines and the time coordinates corresponding either to the commencement or to the end of carbon dioxide administration are made to coincide. The mean ventilation rate on a number of equidistant time coordinates are then calculated and a graph showing the average response drawn.

Figs. 19 and 20 represent the result obtained by superimposing the graphs of six observations on each of the two subjects, I.C. and S.M.G. Superimposition has also been carried out for tidal volume and frequency of respiration. In each figure the graphs are arranged in two sets. Each set consists of three graphs showing separately the effect on ventilation rate, tidal volume and frequency of respiration. The upper set shows the response on administration of carbon dioxide and the lower set the response on its withdrawal.

The average curve for the ventilation rate in subject I.C. (Fig. 19) shows a fairly steep rise between $\frac{1}{2}$ and 1 min. from the beginning of carbon dioxide inhalation and reaches a level of 1·5 litres/

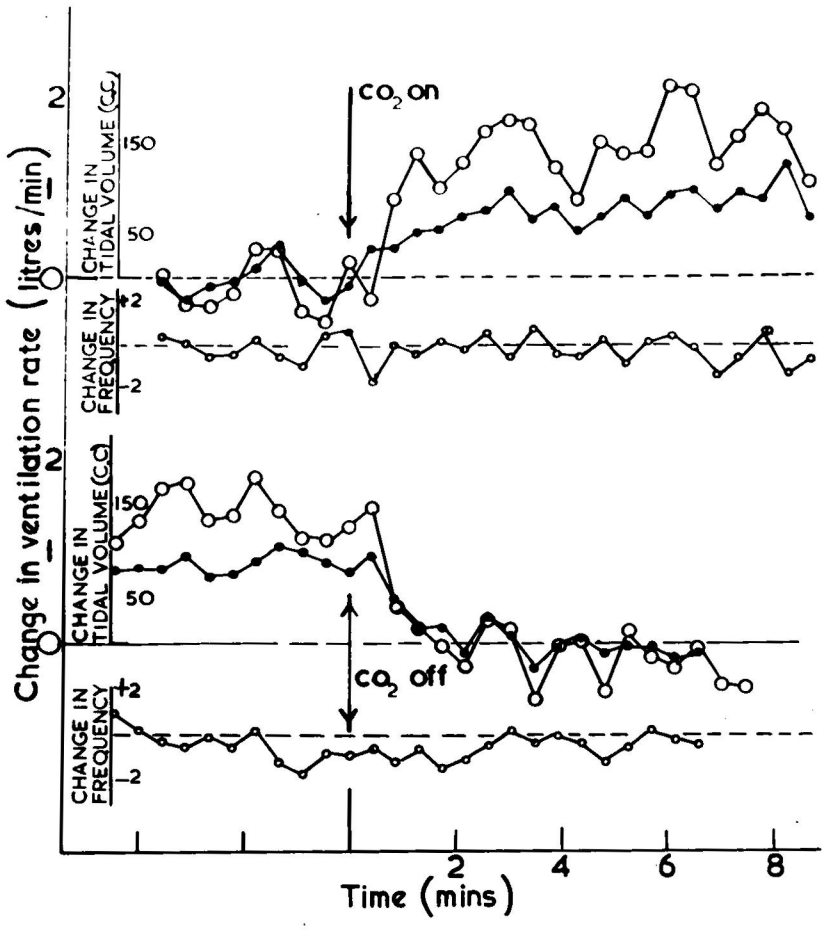

Fig. 19.

The effect of carbon dioxide on total pulmonary ventilation, tidal air, and frequency of respiration. Average of six observations on subject I.C.

o—o—o Total ventilation.

•—•—• Tidal volume.

o—o—o Frequency of respiration.

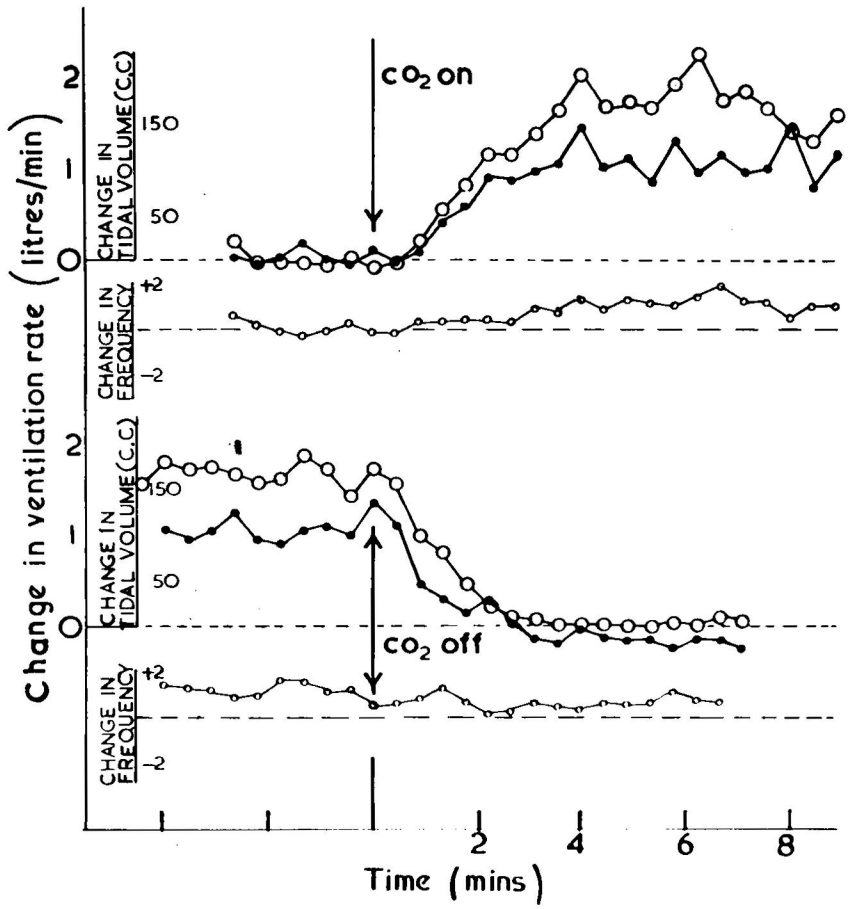

Fig. 20.

The effect of carbon dioxide on total pulmonary ventilation, tidal air, and frequency of respiration. Average of six observations on subject S.M.G.

o—o—o Total ventilation

•—•—• Tidal volume

•—•—o Frequency of respiration

min. above the basal in 3 min. The ventilation rate is then seen to fluctuate at this level during the rest of the period of carbon dioxide inhalation. On discontinuance of carbon dioxide, the ventilation rate returns to its resting level in 2 min.

Compared to the above, the rise and fall in the ventilation rate of subject S.M.G. (Fig. 20) in response to the administration and withdrawal of carbon dioxide seem to occupy a little longer time. The irregularities seen in graphs of single observations (Fig. 17) have almost completely disappeared in the average graph. The ventilation rate in this subject starts rising about ½ min. after the beginning of the carbon dioxide administration and reaches an apparently steady level (1·75 litres/min. above resting level) in 4 minutes. The return to normal level after the discontinuance of carbon dioxide takes about 3 minutes.

Figs. 19 and 20 also demonstrate the effect of carbon dioxide on the tidal volume and frequency of respiration. Carbon dioxide produced an increase of the depth of breathing in both subjects. But there appears to be no certain relation between carbon dioxide inhalation and the rate of respiration. In subject I.C. the rate of respiration was apparently unaffected; in the other subject there was a slight rise. This type of individual variation in the response of respiratory rate to carbon dioxide has been noted before by Barcroft and

Table 7.

RESPIRATORY RESPONSE TO CARBON DIOXIDE ADMINISTERED IN THE INSPIRED AIR AT THE RATE OF 120 c.c./min.

AVERAGE RESULTS OF 6 EXPERIMENTS ON EACH SUBJECT

	Average frequency of respiration per min.	Average volume of tidal air c.c./breath	Average ventilation rate litres/min.
Subject I.C.			
Before administration of CO_2	20	375	7·5
During administration of CO_2 when a steady state has been reached	19·5	455	8·9
After recovery from the effects of CO_2	19·5	375	7·3
Subject S.M.G.			
Before administration of CO_2	11	390	4·25
During administration of CO_2 when a steady state has been reached	12	495	6·0
After recovery from the effects of CO_2	11·3	375	4·25

Margaria (1931).

The average effects produced by carbon dioxide on the ventilation rate, tidal volume and frequency of respiration of the two subjects, as calculated from the graphs in Figs. 19 and 20, are expressed numerically in Table 7.

Administration of carbon dioxide at the rate of 120 c.c./min. to the inspired air increases the ventilation rate of subject I.C. from 7·5 to 9 litres/min., and the tidal volume from 375 to 455 c.c. without any material change in the frequency of respiration. In the second subject, the ventilation rate is increased from 4·25 to 6 litres/min., the tidal volume from 390 to 495 c.c. and the frequency of respiration from 11 to 12.

Elimination of the added carbon dioxide

The effect of administering carbon dioxide to the inspired air on the rate of its elimination in the expired air has been studied in 15 experiments. The data are shown in Table 8 and include observations on all three subjects.

Samples of expired air were examined to determine the resting level of carbon dioxide output of the subject before administration of carbon dioxide. The resting valuesfor the rate of carbon dioxide elimination are shown in column 3 of the table. Subsequent samples were taken over certain periods during or after inhalation of carbon dioxide. Since only four tonometers could be used for

Table 8.

RATE OF ELIMINATION OF THE ADDED CARBON DIOXIDE

	Date / Subject	Rate of CO_2 elimination before CO_2 administration c.c./min.	Rate of elimination of CO_2 in the expired air in excess of its pre-CO_2 administration value in relation to the time of sampling = c.c. of CO_2 per min. / Time since beginning or end of CO_2 administration in minutes			
			During CO_2 administration		After CO_2 administration	
			1st sample	2nd sample	1st sample	2nd sample
1	23.5.54 I.C.	226	$\overline{1}\cdot\overline{42}\frac{82}{-6}\cdot\overline{37}$			
2	14.4.54 S.M.G.	183	$\overline{4}\cdot\overline{70}\frac{102}{-9}\cdot\overline{15}$			
3	28.4.54 S.M.G.	180	$\overline{4}\cdot\overline{47}\frac{120}{-13}\cdot\overline{50}$			
4	10.5.54 I.C.	302	$\overline{3}\cdot\overline{91}\frac{93}{-7}\cdot\overline{90}$	$\overline{7}\cdot\overline{90}\frac{88}{-12}\cdot\overline{20}$		
5	13.5.54 I.C.	228	$\overline{2}\cdot\overline{44}\frac{130}{-8}\cdot\overline{20}$	$\overline{8}\cdot\overline{77}\frac{118}{-14}\cdot\overline{60}$	$\overline{5}\cdot\overline{20}\frac{5}{-9}\cdot\overline{67}$	
6	14.5.54 I.C.	241	$\overline{0}\cdot\overline{31}\frac{79}{-1}\cdot\overline{51}$	$\overline{12}\cdot\overline{8}\frac{132}{-13}\cdot\overline{8}$		
7	15.5.54 I.C.	242	$\overline{0}\cdot\overline{36}\frac{124}{-1}\cdot\overline{64}$	$\overline{3}\cdot\overline{78}\frac{115}{-8}\cdot\overline{20}$	$\overline{0}\cdot\overline{27}\frac{36}{-1}\cdot\overline{69}$	
14	16.6.54 S.M.G.	166	$\overline{0}\frac{\overline{67}}{-6}\cdot\overline{92}$	$\overline{6}\cdot\overline{92}\frac{\overline{127}}{-10}\cdot\overline{80}$	$\overline{0}\frac{\overline{14}}{-3}\cdot\overline{79}$	
15	17.6.54 S.M.G.	188	$\overline{0}\frac{54}{-3}\cdot\overline{84}$		$\overline{0}\frac{30}{-4}\cdot\overline{55}$	$\overline{4}\cdot\overline{55}\frac{-8}{-10}\cdot\overline{10}$

sampling, the number of these subsequent samples was limited to three only.

The figures in the last four columns of Table 8 indicate the average rate of elimination of carbon dioxide expressed as an excess over the pre-carbon dioxide administration rate and also specify the period of sampling. Thus, for example, in experiment 5, the figures $\overset{130}{\underset{2\cdot44\ -\ 8\cdot20}{\ldots\ldots\ldots}}$ indicate that during the interval from 2·44 to 9·20 minute while receiving carbon dioxide with the inspired air, the subject was expiring carbon dioxide at the rate of 228 + 130 c.c. per min.

In some of the samples collected after the end of carbon dioxide administration, the observed elimination rate of carbon dioxide was a little lower than the pre-carbon dioxide-period rate. This is indicated by a negative sign before the relevant figure.

In drawing up this table, it has been tacitly assumed that the rate of metabolic production of carbon dioxide in body of the subject has remained unaltered during the course of the experiment. If there had been variations of a random nature, their effect would tend to cancel out. But if the inhalation of carbon dioxide was to affect metabolism in any particular direction, this would be a systematic source of error. But carbon dioxide is not known to exert any specific influence on metabolism and, according to Shaw and Messer (1930),

adjustment to higher carbon dioxide tension takes place without any effect on the type of metabolism. However, the extra muscular effort necessary to maintain a higher level of pulmonary ventilation theoretically requires extra expenditure of energy. But in the present experiments the ventilatory response was of very low order and the extra muscular effort called for was not expected to increase carbon dioxide production of the body to a degree detectable beyond the limits of error of the method of sampling and analysis employed.

The data contained in Table 8 have been represented graphically in Fig. 21. The extent of the horizontal lines indicates the duration and timing of the period of sampling. The vertical distance of the lines from the abscissa is the average level of carbon dioxide elimination during the period in excess of the pre-carbon dioxide-period elimination rate. The points on the graph occupy the mid-position of the periods of sampling.

The smooth line drawn free-hand give an approximation of how the elimination of carbon dioxide in expired air is likely to behave when carbon dioxide is added to the inspired air at the rate of about 120 c.c./min.

It has been shown already that when the respiration pump was used in the place of a subject, the rate of flow of added carbon dioxide through the apparatus was 115 c.c./min. on the average.

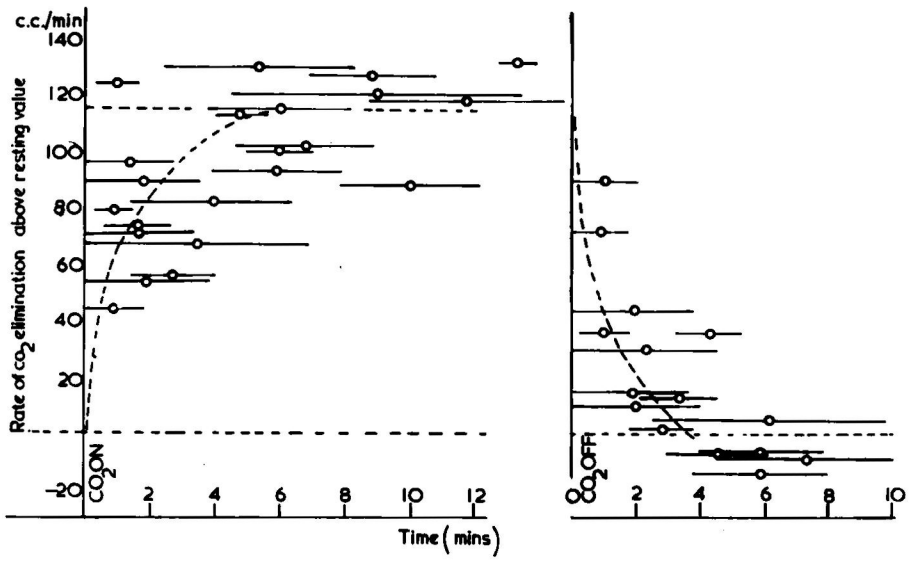

Fig. 21.

The rate of elimination of the added carbon dioxide. The points indicate the average rate over the periods of sampling. The duration and the timing of the period of sampling are shown by the extent of the horizontal lines.

The average of the figures for the extra elimination of carbon dioxide shown in column 5 of Table 8 comes to 113 c.c./min. The graph also shows that after about 4 to 5 minutes the subject eliminates the additional carbon dioxide in the expired air at the rate at which it is added to the inspired air. When carbon dioxide is discontinued, the resting level of its elimination is similarly regained in about 4 minutes.

In the last 6 of the experiments the periods of sampling were without any break in between. From the data of these experiments it is thus possible to prepare something resembling a balance sheet of the amount of carbon dioxide administered to the subject. Such a balance sheet is shown in Table 9. This table also gives a rough estimate of the amount of carbon dioxide which is dammed back within the body during the process of adjustment to the presence of carbon dioxide in the inspired air. Under the conditions of the present experiment, the amount is roughly 120 c.c.

Table 9.

A BALANCE SHEET OF THE CO_2 ADMINISTERED

Serial No. of Expt. as given in Table	DURING INHALATION OF CO_2			AFTER INHALATION OF CO_2 Elimination of the CO_2 which was dammed back		
	Amount of CO_2 administered c.c. CO_2	Amount concurrently eliminated c.c. CO_2	Amount dammed back c.c. CO_2	1st sampling period c.c. CO_2	2nd sampling period c.c. CO_2	Total
10	228	87	141	137	5	142
11	316	263	53	180	31	211
12	422	331	91	54	- 51	3
13	395	246	149	41	- 27	14
14	1255	1077	178	168	-	168
15	411	206	235	138	- 45	93
Total			847			631
Average			141			105

Average estimate of CO_2 dammed back:

From difference during CO_2 inhalation 141

From difference after CO_2 inhalation 105

Mean 123 c.c.

DISCUSSION

The present investigation shows that, if carbon dioxide is administered to the inspired air of a subject at the rate of 120 c.c. per min., his pulmonary ventilation rate starts rising in about ½ min. and reaches a new steady level in 3 to 4 min. On the discontinuance of the carbon dioxide the ventilation rate returns to the original level in 2 to 3 min.

The above inferences are drawn from observations on two subjects only. The graphs in Figs. 19 and 20 show that these inferences are fairly convincing. These graphs represent the average result of 6 observations on each subject. The ventilation curves of single observations were superimposed to calculate the average nature of the ventilatory response to carbon dioxide. This procedure was necessary because of the presence of irregularities in the ventilatory pattern of the subjects. Without this procedure it would have been difficult to decide, for example, in the case of subject I.C., as to when the effect of carbon dioxide on the ventilation rate became steady (compare Figs. 18 and 19).

It appears that if the pulmonary ventilation rate is measured at frequent intervals, a certain degree of irregularity is to be expected in almost any subject. The degree of irregularity varies in

different subjects. But repeated observations on the same subject show that the pattern of irregularity in a given subject tends to be consistent. Why is it so? Pulmonary ventilation is the resultant effect of multiple stimuli acting upon the respiratory centre (Gray, 1946). Is any particular group of stimuli more variable in some subjects than in others or is it the responsiveness of the centre which fluctuates? But these are questions which cannot be answered from the present investigation.

Previous observers who have recorded the rate of pulmonary ventilation in their subjects of experiment at frequent intervals and have tried to estimate the ventilatory response to carbon dioxide, have occasionally referred to the difficulty that may be experienced in deciding when the effect becomes steady. Dripps and Comroe (1947) regarded the ventilation rate of the subject to have reached an apparent plateau if the ventilation rate did not vary by more than 10% during four consecutive 30 second periods. Judged by this standard, only 27 out of 42 subjects of the series in which the

'slightly elastic'. It is felt that the method adopted in the present work to discern the effect of carbon dioxide on the ventilation rate by superimposition of a number of ventilatory records is preferable to an arbitrary standard of defining a plateau.

It has been mentioned earlier that Padget (1928), Dripps and Comroe (1947), Lambertsen et al (1952) and Duncan Weatherley (1952) have found or considered that the ventilatory response to carbon dioxide attains a maximum in a few minutes.

The data of a certain experiment of Campbell et al (1913) have been quoted on page 77 . According to them, the ventilation rate in their subject did not become steady throughout the period of observation of 20 minutes. But an inspection of the actual data will show that the ventilation rate did not practically vary by more than 10% after the 4th or 5th minutes, and so, according to the standards of Dripps and Comroe (1947) and Duncan Weatherley (1952), the ventilatory response in this subject, also, had attained a relative constancy in a few minutes.

The evidence in general, therefore, indicates that the ventilatory response to carbon dioxide may not take a long time to reach its maximum even when the air mixture contains 10% of carbon dioxide. In the experiments of Dripps and Comroe (1947) the ventilatory response has been stated to have

reached a plateau in 2·5 to 6 minutes. The air mixture contained 10% carbon dioxide.

But other experiments of Campbell et al (1913) and Campbell et al (1914), and the experiments of Adolph et al (1928), in which the effect of carbon dioxide inhalation on the rate of elimination of metabolic carbon dioxide from the body has been studied, have shown that the discharge of metabolic carbon dioxide from the body is retarded for a much longer time. It seems very probable that in many of the investigations in which a subject has been made to breathe air mixture enriched with carbon dioxide, his ventilation rate has attained a steady higher level and yet the rate of elimination of the metabolic carbon dioxide from his body has been suffering a retardation. The significance of this state is explained below.

Addition of carbon dioxide to the inspired air of a subject temporarily disturbs the steady state of carbon dioxide equilibrium in him. When so disturbed there are theoretically two possible ways by which the steady state can be regained. These two means of adjustment are:

1) An increase in the partial pressure of alveolar carbon dioxide.
2) An increase in pulmonary ventilation.

The second of these two means is a physiological process depending on the integrity and responsiveness of the neuromuscular mechanism which controls

the respiratory movements. The purpose of the physiological mechanism is essentially to limit the other means of adjustment, i.e. to prevent any material increase in the partial pressure of alveolar carbon dioxide.

In experimental animals, anaesthetized and ventilated artificially, the neuromuscular mechanism controlling the respiration becomes inoperative. If such an animal preparation is ventilated with an air mixture containing a high percentage of carbon dioxide, as has been done in the experiments of Shaw (1928), Shaw and Messer (1930) and Irving et al (1930), the alveolar concentration of carbon dioxide is raised much above its normal value. The diffusion gradient of carbon dioxide across the alveolar membrane may at first be completely reversed and the respiratory quotient may become negative (Irving et al, 1930). The carbon dioxide produced in the body of the animal accumulates in the tissues till the tension rises sufficiently to re-establish a diffusion gradient in the proper direction and of the right magnitude. When this happens carbon dioxide is again eliminated from the animal body and the rate of its production and the respiratory quotient returns to normal. But the time required to attain the new state of equilibrium is very prolonged. In cats it may take a couple of hours before the respiratory quotient returns to normal. The amount of carbon dioxide retained in the body of cats under such conditions was found to

be so enormous by Irving et al (1930) that they were led to consider the possibility of the bones participating in the process.

These are extreme examples and the experimenters had entirely different objects in view. But they serve to illustrate what is going to happen in intact animals or human subjects if their pulmonary ventilation capacity is overtaxed by the administration of air mixtures containing high percentages of carbon dioxide. The ventilation rate may then fail to rise to the extent necessary for the full elimination of the endogenous carbon dioxide which will therefore continue to accumulate partially in the body for a long time after the ventilation rate has reached an apparent plateau. This seems to be the explanation of how it has been reported by some observers - that the ventilation rate reaches a plateau in a few minutes, and yet Campbell et al (1913), Campbell et al (1914) and Adolph et al (1928) found evidence of the retention of carbon dioxide for a very much longer time.

Therefore, if one intends to study the ability of the respiratory mechanism to deal with an excess of carbon dioxide, it is desirable that the ventilatory response and the rate of elimination of carbon dioxide should both be followed. This has been the line of approach of the present study.

Having found that the ventilatory response reached a steady level in 3 to 4 minutes, an

attempt was made to find out how soon the rate of elimination of carbon dioxide in the expired air accounted for the amount added to the inspired air. It is to be admitted that the method adopted for following the rate of elimination of carbon dioxide in the expired air was not as satisfactory as the method by which the ventilatory response was followed. The method of sampling the expired air was limited to a maximum of four samples. It was only approximately possible to make the rate of sampling proportionate to the rate of flow of expired air at the site of sampling. In spite of all these limitations the graphical representation of the observed data on the rate of elimination of the additional carbon dioxide in Fig. 21 indicates the general trend of a carbon dioxide balance being attained at about the same time at which the ventilatory response in the present experiments usually reached a steady state.

There is now almost general agreement that the main site of the action of carbon dioxide in producing hypernoea is the respiratory centre (Schmidt and Comroe, 1940). In order to produce and maintain the hypernoea, a certain amount of carbon dioxide has to accumulate in the body. The experiments of Campbell et al (1913) and Campbell et al (1914) demonstrated that an amount of carbon dioxide accumulated within the body during inhalation of air enriched with carbon dioxide. It

is possible to calculate from the experimental data of Adolph et al (1928) that about 180 c.c. of carbon dioxide accumulated in the body per mm. of mercury rise in the pressure of alveolar carbon dioxide. But it has been discussed already that these experimenters were probably dealing with a state in which the capacity of physiological adjustment had been exceeded. It could be argued that the accumulation of carbon dioxide under such conditions was the result of the inadequacy of the physiological response rather than its necessary accompaniment.

The present investigation has been carried out within the physiological capacity of respiratory adjustment. The amount of carbon dioxide added to the inspired air was small. The rate of addition was fixed and not its percentage, so its concentration in the inspired air depended on the ventilation rate. For example, in subject I.C., when his ventilation rate adjusted to the administration of carbon dioxide, its percentage in the inspired air was only 1·3%. The fact that the ventilatory response and the rate of elimination of carbon dioxide in the expired air reached the new steady state almost simultaneously justify the conclusion that the physiological response was adequate. The demonstration that an amount of carbon dioxide does accumulate within the body under these conditions is, therefore, significant and support the generally accepted idea that an amount of carbon dioxide must

accumulate within the body to maintain the hyperpnoea of carbon dioxide inhalation.

The average amount of carbon dioxide dammed back in the body during the present experiments have been found to be roughly 120 c.c. If the dammed back carbon dioxide is considered to have been uniformly distributed in the aqueous media of the body, and the intracellular fluid be regarded to be equivalent as regards carbon dioxide capacity to one-third its volume of extracellular fluid or blood, then the carbon dioxide tension of the tissues had been increased by 1 mm. of mercury due to the retained carbon dioxide in these experiments.

Campbell et al (1914) have estimated that a rise of 2·0 mm. of mercury in the tension of alveolar carbon dioxide produces an increase of 10 litres in the total lung ventilation. According to Nielsen and Smith (1951), the carbon dioxide 'sensitivity index' of the respiratory centre corresponds to an increase of 2 litres in the ventilation rate per mm. of mercury increase in the carbon dioxide tension. In calculating the so-called 'sensitivity index', Nielsen and Smith (1951) have divided the actually observed increase in the ventilation rate by the observed rise in the alveolar tension. Campbell et al (1914) did not actually measure the ventilatory response but calculated its expected value on the assumption that the subject had reached the steady state of

carbon dioxide equilibrium. There is no definite assurance that a steady state was reached in the experiments of either of the two groups of workers.

In the subjects of the present investigation the average increase in the pulmonary ventilation amounted to 1·75 litres/min. in one subject, and 1·5 litres/min. in another (Table 7). This increase in the ventilation rate was apparently adequate for restoring the steady state of carbon dioxide equilibrium in the subjects. The process of adjustment was complete in 3 to 4 minutes, and was accompanied by an accumulation of about 120 c.c. of carbon dioxide in the body. The retained carbon dioxide uniformly distributed in the aqueous phase of the body would be sufficient to raise the tension of carbon dioxide by about a millimetre. If this had been so, the carbon dioxide sensitivity of the respiratory centre in these subjects was definitely lower than the value estimated by Campbell et al (1914). The possibility exists that a part of the retained carbon dioxide found its way into the bones, in which case the rise in carbon dioxide tension would have been less than one millimetre and the value of the 'sensitivity index' correspondingly high. The possibility cannot be examined without a more accurate estimate of the amount of carbon dioxide dammed back within the body than has been made in the present investigation.

SUMMARY AND CONCLUSIONS

A method is described for measuring the effects of breathing carbon dioxide under conditions resembling those of an increased production of endogenous carbon dioxide.

The respiratory response to carbon dioxide administered in the inspired air at the rate of 120 c.c./min., and to its withdrawal have been studied mostly in two subjects.

Under the conditions of the present study, the respiratory response to carbon dioxide has been found to reach a steady state in 3 to 4 minutes following its administration, and in 2 to 3 minutes following its withdrawal.

An amount of carbon dioxide is dammed back within the body when a subject inhales carbon dioxide in air, and this is given out later when he breathes pure air. In the present experiments the amount of carbon dioxide retained and later given out have been roughly found to be 120 c.c.

The significance of the retention of carbon dioxide and its relation to carbon dioxide sensitivity of the respiratory centre are discussed.

ABSTRACT

Carbon dioxide is not to be considered simply as an excretion to be voided from the body as rapidly as it is formed. On the contrary, it is an essential constituent of the internal environment and, like the other ultimate product of metabolism, water, its concentration in the body is adjusted by finely balanced mechanisms. This work is concerned with certain aspects of the distribution and control of body carbon dioxide.

The first part deals with the diffusion of carbon dioxide in fats. The diffusion coefficient and solubility of the gas in animal fat have been determined and the results applied to the question of the movement and distribution of carbon dioxide in body fat. It is concluded that the distribution of carbon dioxide in fat and the time factors involved in its movement can account for only a small fraction of the quantity involved in the process of equilibration following an altered pressure of carbon dioxide in the lungs.

The second part is concerned with the exchange of carbonate between bones and surrounding fluids. Experiments with frog bones are described, the results of which indicate that in the bones there exist anion positions for which phosphate and bicarbonate ions can compete. It is discussed how far various reports in the literature on the

lability of bone carbonate also imply an anion exchange, and it is concluded that this is a probable mechanism by which bone carbonate may participate in exchanges with body fluids and possibly assist in the regulation of their carbon dioxide concentration.

In the third part a method is described for measuring the effects of breathing carbon dioxide under conditions resembling those of an increased production of endogenous carbon dioxide. Carbon dioxide was administered in the inspired air at a fixed rate of approximately 120 c.c./min. so as to influence the composition of the alveolar air in a manner similar to an increase of the resting metabolism by roughly 50%. It was found that the ventilation rate and the elimination of carbon dioxide in the expired air reached a steady state in about 4 minutes. In the process of attaining the new steady state about 120 c.c. of carbon dioxide was retained within the body. The significance of the retention of carbon dioxide and its relation to carbon dioxide sensitivity of the respiratory centre are discussed.

REFERENCES

Adolph, E.F., Nance, F.D. & Shilling, M.S. (1928). The carbon dioxide capacity of the human body and the progressive effects of carbon dioxide upon the breathing. Amer. J. Physiol. 87, 532-541.

Andrews, D.H. & Johnston, J. (1924). The rate of absorption of water by rubber. J. Amer. Chem. Soc. 46, 640-650.

Barcroft, J. & Margaria, R. (1931). Some effects of carbonic acid on the character of human respiration. J. Physiol. 72, 175-185.

Basset, H.J. (1917). The phosphates of calcium. Part IV. The basic phosphates. J. Chem. Soc. 111, 620-642.

Campbell, J.M.H., Douglas, C.G., Haldane, J.S. & Hobson, F.G. (1913). J. Physiol. 46, 301-318.

Campbell, J.M.H., Douglas, C.G. & Hobson, F.G. (1914). The sensitiveness of the respiratory centre to carbonic acid and the dead space during hypernoea. J. Physiol. 48, 303-315.

Cross, K.W. (1949). The respiratory rate and ventilation in the newborn baby. J. Physiol. 109, 459-474.

Davidson, D., Eggleton, P. & Foggie, P. (1952). The diffusion of atmospheric gases through fats and oils. Quart. J. exp. Physiol. 37, 91-105.

Davies, H.W., Brow, G.R. & Binger, C.A.L. (1925). The respiratory response to carbon dioxide. J. exp. Med. 41, 37-52.

Daynes, H.A. (1920). The process of diffusion through a rubber membrane. Proc. Roy. Soc. London. A. 97, 286-307.

Dickens, F. (1941). The citric acid content of animal tissues, with reference to its occurrence in bone and tumour. Biochem. J. 1011-1023.

Dripps, R.D. & Comroe, J.H.,Jr. (1947). The respiratory and circulatory response of normal man to inhalation of 7·6 and 10·4 per cent CO_2 with a comparison of the maximal ventilation produced by severe muscular exercise, inhalation of CO_2 and maximal voluntary hyper-ventilation. Amer. J. Physiol. 149, 43-51.

Duncan-Weatherley, A.H. (1952). The ventilatory response to low concentrations of carbon dioxide in normal young men. Aust. J. exp. Biol. med. Sci. 30, 95-104.

Eggleton, G.P., Eggleton, P. & Hill, A.V. (1928). The coefficient of diffusion of lactic acid through muscle. Proc. Roy. Soc. B. 103, 620-628.

Eggleton, M.G. (1933). Diffusion of inorganic phosphate into and out of the skeletal muscles and bones of the frog. J. Physiol. 79, 31-48.

Eggleton, P. (1930). The diffusion of creatine and urea through muscle. J. Physiol. 70, 294-300.

Eggleton, P. (1952). Diffusion phenomenon in biology. In Deformation and Flow in Biological Systems. ed. Frey Wyssling, A. Amsterdam: North-Holland.

Eggleton, P., Elsden, S.R., Fegler, J. & Hebb, C.O. (1945). A study of the effects of rapid 'decompression' in certain animals. J. Physiol. 104, 129-150.

Falkenheim, M., Neuman, W.F. & Hodge, H.C. (1947). Phosphate exchange as the mechanism for adsorption of the radioactive isotope by calcified tissues. J. biol. Chem. 169, 713-722.

Fenn, W.O. (1928). The carbon dioxide dissociation curve of nerve and muscle. Amer. J. Physiol. 85, 207-223.

Ferguson, J.K.W., Irving, L. & Plewes, F.B. (1929). The source of expired CO_2 in decapitated eviscerated cats. J. Physiol. 68, 165-276.

Fiske, C.H. & Subbarow, Y. (1925). The colorimetric determination of phosphorus. J. biol. Chem. 66, 375-400.

Fiske, C.H., Goodell, R.A., Hathaway, L.,Jr, & West, E.J. (1926). Further observations on the fate of acid in the body. J. biol. Chem. 67, 385-396.

Freeman, F.H. & Fenn, W.O. (1953). Changes in carbon dioxide stores of rats due to atmospheres low in oxygen or high in carbon dioxide. Amer. J. Physiol. 174, 422-430.

Gersh, I. & Still, A.M. (1945). Blood vessels in fat tissue. Relation to problems of gas exchange. J. exp. Med. 81, 219-232.

Goto, K. (1918). Mineral metabolism in experimental acidosis. J. biol. Chem. 355-376.

Gray, J.S. (1946). The multiple factor theory of the control of respiratory ventilation. Science. 103, 739

Hagenbach, A. (1898). Ueber Diffusion von Gasen durch Wasserhaltige Gelatine. Ann. Phys., Lpz. 65, N.S. 673-706.

Haldane, J.S. & Priestley, J.G. (1905). The regulation of lung ventilation. J. Physiol. 32, 224-266.

Hasting, A.B. (1940). The electrolytes of tissues and body fluids. The Harvey Lectures. 36, 91-125.

Henderson, L.J. (1928). Blood, A study in general physiology. 1st ed. Newhaven: Yale University Press.

Hendricks, S.B., Hill, W.L., Jacob, K.D. & Jefferson, M.E. (1931). Structural characteristics of apatite-like substances and composition of phosphate rock and bone as determined from microscopical and X-ray diffraction examinations. Industrial and Engin. chem. 23, 1413-1418.

Hendricks, S.B., Jefferson, M.E. and Mosley, V.M. (1932). The crystal structures of some natural and synthetic apatite-like substances. Ztsch. f. Kristall. 81, 352-369.

Hill, A.V. (1928). The diffusion of oxygen and lactic acid through tissues. Proc. Roy. Soc. London, B. 104, 39-96.

Hodge, H.C., Neuman, W.F., Osterhoudt, J., Mulryan, B.J. & Bale, W.F. (1953). The nature of the carbonate of bone mineral. XIX International Congress. Montreal. Abstracts and Communications, p. 467.

Holt, L.E., La Mer, V.K. & Chown, H.B. (1925). Studies in calcification. The solubility product of secondary and tertiary calcium phosphate under various conditions. J. biol. Chem. 64, 509-564.

Howland, J., Marriott, W.M. & Kramer, B. (1926). Studies upon the inorganic composition of bones. J. biol. Chem. 68, 721-728.

Hufner, G. (1897). Ueber die Bestimmung der Diffusioncoefficienten einiger Gase fur Wasser. Ann. Phys., Lpz. 60, N.S. 134-168.

Huggins, C. (1937). The composition of bone and function of bone cell. Physiol. Rev. 119-143.

Irving, L. and Chute, A.L. (1932). The participation of carbonates of bone in the neutralization of ingested acid. J. cell. comp. Physiol. 2, 157-176.

Irving, L., Ferguson, J.K.W. & Plewes, F.B. (1930). The source of CO_2 expired and the site of its retention. J. Physiol. 69, 113-123.

Johansson, E.G., Falkenheim, M. & Hodge, H.C. (1945). The adsorption of phosphates by enamel,dentin and bone. Adsorption times studied by means of radioactive isotopes. J. biol. Chem. 159, 129-134.

Keys, A. & Brozek, J. (1953). Body fat in adult man. Physiol. Rev. 33, 245-325.

Krogh, A. (1919). The rate of diffusion of gases through tissues, with some remarks on the coefficient of diffusion. J. Physiol. 52, 390-408.

Lambertsen, C.J., Kough, R.H., Cooper, D.Y., Emmel, G.L., Loeschcke, H.H. & Schmidt, C.F. (1952). Comparison of the relationship of respiratory minute volume to pCO_2 and pH of arterial and internal jugular blood in normal man during hyperventilation produced by low concentration of CO_2 at 1 atmosphere and by O_2 by 3 atmosphere. J. appl. Physiol. 5, 803-813.

McCance, R.A. & Widdowson, E.M. (1951). A method of breaking down the body weights of living perons into terms of extracellular fluid, cell mass and fat, and some applications of it to physiology and medicine. Proc. Roy. Soc. B, 138, 115-130.

Marriott, H.L. (1947). Water and salt depletion. Brit. med. J. 1, 245

Morgulis, S. (1931). Studies in the chemical composition of bone ash. J. biol. Chem. 93, 455-466.

Neal, W.M., Palmer, L.S., Eckles, C.H. & Guillickson, T.W. (1931). Effect of age and nutrition on the calcium phosphate calcium carbonate ratio in the bones of cattle. J. agric. Res. 42, 115-121.

Neuman, W.F. & Mulryan, B.J. (1951). The surface chemistry of bone. IV. Further data on re-crystallization. J. biol. Chem. 193, 237-241.

Neuman, W.F., Neuman, M.W., Main, E.R., O'Leary, I. and Smith, F.A. (1950). The surface chemistry of bone. II. Fluoride deposition. J. biol. Chem. 187, 655-661.

Newton, W.H. (1939). Recent Advances in Physiology, 6th ed. p.22. Longon: Churchill.

Nielsen, M. & Smith, H. (1951). Studies on the regulation of respiration in acute hypoxia. Acta. physiol. Scand. 24, 293.

Padget, P. (1928). The respiratory response to carbon dioxide. Amer. J. Physiol. 83, 384-387.

Peabody, F.W. (1915). Clinical studies on the respiration. No. 1. The effect of carbon dioxide in the inspired air on patients with cardiac disease. Arch. Intern. Med. 16, 846-864.

Robinson, R., Macleod, M. & Rosenheim, A.H. (1930). The possible significance of hexose phorphoric esters in ossification. IX. Calcification in vitro. Biochem. J. 24, 1927-1941.

Roseberry, H.H., Hastings, A.B. & Morse, J.K. (1931). X-ray analysis of bone and teeth. J. biol. Chem. 90, 395-406.

Roughton, F.J.W. (1935). Recent work on carbon dioxide transport by the blood. Physiol. Rev. 15, 241-296.

Roughton, F.J.W. (1943). Some recent work on the chemistry of carbon dioxide transport by the blood. The Harvey Lectures. 39, 96-142.

Schaffer, P.S. & Haller, H.S. (1943). The solubility of gases in butter oil, cotton seed oil and lard. Oil and Soap. 20, 161.

Schmidt, C.F. & Comroe, J.H.,Jr. (1940). Functions of the carotid and aortic bodies. Physiol. Rev. 20, 115-157.

Shaw, L.A. (1928). The comparative capacity of the blood and of the tissue to absorb carbonic acid. Amer. J. Physiol. 79, 91-99.

Shaw, L.A. & Messer, A.C. (1930). The carbon dioxide capacity of the body and the rate at which the body comes into equilibrium with changes in alveolar carbon dioxide tension. Amer. J. Physiol. 93, 422-432.

Shear, M.J. & Kramer, B. (1928). Composition of bone. 1. Analytical micromethod. J. biol. Chem. 79, 105-120.

Shear, M.J. & Kramer, B. (1928). Composition of bones. IV. Primary calcification. J. biol. Chem. 79, 147-160.

Shohl, A.T. (1939). Mineral Metabolism. (American Chemical Society Monograph. 82), 1st ed. p. 32. New York: Reinhold.

Sobel, A.E., Rockenmacher, M. & Kramer, B. (1944). Microestimation of the inorganic constituents of bone. J. biol. Chem. 152, 255-266.

Sobel, A.E., Rockenmacher, M. & Kramer, B. (1945). Carbonate composition of bone in relation to the composition of blood and diet. J. biol. Chem. 158, 475-489.

Van Slyke, D.D. (1926). Factors affecting the distribution of electrolytes, water, and gases in the animal body. Philad: London: Lippincott.

Van Slyke, D.D. & Neil, J.M. (1924). The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. J. biol. Chem. 61, 523-573.

Van Slyke, D.D., Sendroy, J.,Jr., Hastings, A.B., & Neil, J.M. (1928). Studies of gas and electrolyte equili bria in blood. X. The solubility of carbon dioxide at 38°C. in water, salt solution, serum and blood cells. J. biol. Chem. 78, 765-799.

Van Slyke, D.D., Sendroy, J.,Jr. & Liu, S.H. (1932). Manometric analysis of gas mixtures. II. Carbon dioxide by the isolation method. J. biol. Chem. 95, 531-546.

Vibrans, F.C. (1935). Solubility of gases in edible fats and oils. Oil and Soap. 12, 14-15.

Vogt, J.H. & Tønsager, A. (1948). Investigations on the bone chemistry of man. II. Acta med. Scand. 135, 231-244.

Wallace, W.M. & Hastings, A.B. (1942). The distribution of bicarbonate ion in mammalian muscle. J. biol. Chem. 144, 637-649.

Wright, C.I. (1934). The diffusion of carbon dioxide in tissues. J. gen. Physiol. 17, 657-676.

Addendum to References

Conner, L.A. & Stillman (1911). A pneumographic study of respiratory irregularities in meningitis. Transactions of the Association of American Physicians. 26, 464-485.

Greene, J.A. (1933). Clinical studies of respiration. IV. Some observations on Cheyne-Stokes respiration. Arch. intern. Med. 52, 454-463.

Greene, J.A. & Coggeshall, H.C. (1933). Clinical studies of respiration. I. Plethysmographic study of quiet breathing and of the influences of some ordinary activities on the expiratory position of the chest in man. Arch. intern. Med. 52, 44-56.

Mosso, A. (1886). La respiration périodique et la respiration superflue on de luxe. Arch. ital. Biol. 7, 48-127.

Mosso, A. (1903). Les mouvements respiratoires du thorax et du diaphragme. Arch. ital. Biol. 40, 43-98.